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THE VARIABILITY OF PLANTS GROWN IN WATER CULTURES[1]

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During recent years, much emphasis has been placed upon the water-culture method as a means of determining the influence of salts upon plant growth. Because of its relative simplicity of composition and susceptibility to control, it has proved an especially valuable procedure in certain fields of agricultural research. Like other methods, however, it is not ideal. It possesses certain inherent errors which, if not recognized, may lead to a misinterpretation of data. In this study, it is desired especially to emphasize the extensive rôle plant variation may assume, even under the "controlled" conditions usually insisted upon in experiments involving culture solutions.

Most of the work of this sort involves the comparison of many diverse solutions, and where the relative salt requirements of plants are under consideration, such solutions may differ but little from one another. Where this is true, the results usually vary similarly, and the question arises as to whether fixed values can be assigned to differences of such degree when the material being compared shows extensive variation.

The general practice has been to base conclusions upon relatively few plants. Thus cultures, which as a rule contain 1 to 6 plants, are usually set up in duplicate, and the "growth efficiency" of solutions compared through the mean dry weights. Brenchley (1) in a report on the effect of varying concentrations of copper sulfate and manganese sulfate upon the growth of barley, employed the mean of 20 replicate cultures of one plant each in her comparisons, while in later work (2) the mean of 10 plants was used. Stiles (11), studying the effect of different salt concentrations upon the growth of barley, rye, and wheat, likewise based conclusions upon the mean of 10 replicate cultures of one plant each. Tottingham (13), comparing the relative efficiency of many modifications of Knop's solution differing in total as well as in partial salt concentration, employed duplicates with 6 plants to a culture, Shive (7), following him in another phase of the same general investigation, set up single cultures of 6 plants each, and after several weeks repeated the experiment; corresponding cultures of the two series were then averaged, the mean dry weights being taken for comparison. Later Shive and Martin (10)

[1] This study was undertaken at the instance, and under the direction, of Dr. C. B. Lipman. The writer is indebted also to Dr. Geo. A. Linhart for kindly criticism, and to Dr. D. D. Waynick for suggestive preliminary work.

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followed this same general procedure in working with young and mature buckwheat. For the mature phase, however, many more cultures than were required for the experiment were grown to the end of the "young" phase in a solution which had proved favorable, and from this excess the necessary number of cultures, uniform apparently insofar as the eye could judge, were selected for the second stage of the work. There are many other investigations which might be cited on this point, but these serve to illustrate the common practice.

The well established fact of biological variation under water-culture conditions has not been ignored, rather various expedients have been employed to minimize it. Thus the rotating table was employed by Shive (7) and others as a means of equalizing environmental influences. Seedlings have been very generally selected for uniform size and vigor and in certain cases [Brenchley (1)] seeds have been graded according to weight. Cultures have usually been made up of several plants, the idea being that less vigorous plants would offset more vigorous ones, and the total weight of any culture would not depart significantly from its replicates. In spite of such precautions, however, variability remains an important factor. Both Brenchley (1) and Stiles (11, 12) recognized that the number of plants which they employed was insufficient to give a mean of more than low accuracy, the former remarking that "owing to the individuality of plants, investigations dealing with small numbers cannot be depended upon," and the latter that, "plants growing in water cultures under exactly the same conditions are very variable." Both of these workers determined the probable error of the mean and interpreted results accordingly, but because of the limited number of determinations averaged and their rather high variability (40 per cent in Brenchley's work), the accuracy of this probable error is very questionable.

American workers have tacitly assumed that under the system of control adopted, variation is reduced to a negligible minimum; in other words, that the mean of few determinations will not depart sufficiently from that of many to vitiate conclusions. One need but examine the literature, however, to find many instances contrary to such an assumption. In many cases noted, where duplicate cultures have been employed, wide differences between them were apparent; in fact, such differences have been frequently observed to exceed that existing between the means under comparison. Where this is true, how much trust can we place in the mean? Certainly, under such conditions, means of duplicates cannot possess any high degree of accuracy, and differences attributed to efficiency of the solution may be due to variability of the plants themselves.

The first consideration, then, in comparing such variables as plants, is the determination of the trustworthiness of the mean; its limit of accuracy must be fixed. This will demand the consideration of a much larger number of replicates than workers have deemed necessary heretofore. Since the distribution of biological variables has been found to follow closely the law of prob-

ability, it is evident that the larger the number of variables averaged, the more accurate will be the arithmetic mean.[2] On the contrary, small numbers will give means which may depart more or less widely from the true mean, the extent of departure depending on the efficiency of the system.

In the statistical study herein reported, Shive's (7) extended work is taken as a basis. This was selected on account of the very careful attention the author gave to the many details, as well as because it considered the critical comparison of several solutions. Shive employed his well known three-salt solution in three different total concentrations, 0.1, 1.75 and 4.00 atmospheres; (the second of these, the 1.75-atmosphere concentration, is considered here). In these the partial molal concentration of salts was varied in increments of one-tenth, 36 different combinations resulting. A series of 36 cultures, each culture representing different partial concentrations of salts, was set up in 250-cc. bottles, the solutions changed every 3 days, and the experiment continued for 23 days on a revolving table. The greatest care seems to have been taken to have the culture solution the only condition of the environment differing in its influence upon the individual cultures. A careful perusal of the paper did not disclose whether duplicate cultures were set up or not. However, the experiment was repeated at a later date and the means of the dry weights of corresponding cultures in the two series taken for comparison of the efficiency of the solution. Results were plotted by the familiar triangle method as employed in physical chemistry, and on this triangle, areas of high and low yields were mapped out. Lying outside such areas were many results which might be considered medium in their value and these are especially important in a consideration of the data as a whole.

METHODS

In any study of frequency distribution, it is extremely desirable to have a sufficient number of variants to permit of an approximately normal distribution about the mean. In this study, we are dealing only with Shive's 1.75-atmosphere solution, and yet this alone contains 36 different combinations. It would manifestly be difficult to consider all of these statistically, nor is it necessary, since, in order to demonstrate the principle involved, one needs but to consider a few well chosen culture solutions of the 36 represented. For our work, modifications R_5C_2, R_2C_5, and R_1C_1 were chosen, these representing his so-called "best," his "poorest," and a "medium" solution,[3] the last named lying between the highest and lowest-yield areas. These three had the following partial molal concentrations:

[2] This, however, as pointed out by Linhart (4), can be true only where the maximum deviation does not exceed the mean in magnitude.

[3] The designations are those employed by Shive.

	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
R_5C_2	0.0180	0.0052	0.0150
R_2C_5	0.0072	0.0130	0.0150
R_1C_1	0.0036	0.0026	0.0400

It is likewise true that the number of replicate cultures possible to consider in an experiment of this kind is limited by one's time and equipment, since the changing of solutions each three days for a large number of cultures is a considerable task. For our purposes, two series, "A" and "B", were set up, the first in quart Mason jars and the latter in 250-cc. bottles. Thirty-three cultures (165 plants) for each of the three culture solutions were arranged for Series "A" and 50 (300 plants) for Series "B." Such numbers, of course, were purely arbitrary, but it was felt they would be sufficient for the purpose at hand.

Culture solutions

Baker's analyzed chemicals were employed throughout. Stock solutions of single salts were made up and from these dilutions were made sufficient for a single change. Throughout this work, the importance of subjecting all cultures in any one series to the same culture-solution environment was emphasized and to this end the total amount of solution required for the 33 or 50 cultures, as the case might be, was made up in one lot and this thoroughly mixed several times before being placed in the final containers.

Seedlings

Instead of the Fulcaster wheat used by Shive, Tottingham, McCall, and others, Sonora wheat of selected strain and high germination was employed. This was obtained from the Division of Agronomy of the University of California. Shive's method of germination was used, many more seedlings than necessary being germinated, and only uniform, vigorous seedlings selected for cultures. This latter point was especially stressed, each seedling being carefully measured and all those not coming within the limits of 5 $\pm$ 1 cm. rejected.

Glassware

As stated above quart Mason jars were used for series A and 250-cc. wide-mouth bottles for series B. These were cleansed with chromic-sulfuric cleaning mixture, thoroughly rinsed and covered with opaque paper, white side out. Corks were given a very thin coating of "parowax."

Setting up experiments and changing solutions

The two series were set up in the greenhouse, series A on long benches, the position of the jars being changed several times a week in accordance with a

definite plan; series B was placed on revolving tables. Light conditions were excellent and the jars were arranged so as to prevent the shading of one culture by another. Likewise the jars were sufficiently removed from each other to obviate unequal inter-humidity effects. The unheated condition of the greenhouse gave rise to slightly higher variations in day and night temperatures than would have been true had heat been applied (table 1), but such differences were not significantly greater than those reported by Shive and since they affected all plants alike, they were not held to be important.

TABLE 1

Temperatures

	SERIES "A"	SERIES "B"
	°C.	°C.
Mean maxima	26.8	20.8
Maximum	32.5	29.0
Mean minima	9.4	5.2
Minimum	5.5	1.0

In changing solutions, the following procedure was adopted. The total amount of solution required for a single modification in series A, say for R_5C_2, was 33 liters. This amount was made up from the strong single-salt stock solutions and thoroughly shaken. The cork and plants were placed in an extra bottle containing the solution, the old solution emptied out of the jar and the new lot measured in. The plants were then replaced. In series B the total amount for a change was about 12.5 liters. This was made up in a similar way and the change performed as above. The amount of transpiration was ascertained by measuring the old solution before discarding it.

Criterion of growth

Dry weight of the entire plant and of the top alone was taken as the criterion of growth. After harvesting, the plants were dried at 90°C. for 48 hours, and then to constant weight at 105°C. The weighings for series A were made to 0.01 and in series B to 0.001 gm.

Calculations

The statistical method presented by Davenport (3) and in general use in biometry, was employed except for the calculation of theoretical curves. The latter were calculated according to an equation recently obtained by Dr. G. A. Linhart. Since these methods are taken up *in extenso* by Davenport, by Dr. Linhart (4), and in various texts, it is not thought necessary to go into a mathematical treatment of them here.

TABLE 2

*Total weights, series A**

NUMBER	R_5C_2 Weight	R_5C_2 Deviation from mean	R_2C_5 Weight	R_2C_5 Deviation from mean	R_1C_1 Weight	R_1C_1 Deviation from mean
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	2.64	0.49	2.36	0.22	2.00	0.44
2	2.50	0.35	2.33	0.19	1.57	0.01
3	1.92	0.23	1.96	0.18	1.64	0.08
4	2.06	0.09	2.05	0.09	1.57	0.01
5	2.00	0.05	1.66	0.48	1.76	0.20
6	2.13	0.02	2.47	0.33	1.61	0.05
7	2.09	0.06	1.99	0.15	1.38	0.17
8	2.34	0.19	1.94	0.20	1.53	0.03
9	2.24	0.09	2.33	0.19	1.34	0.22
10	2.06	0.09	2.02	0.12	1.34	0.22
11	2.18	0.03	2.13	0.01	1.57	0.01
12	2.18	0.03	2.13	0.01	1.40	0.16
13	2.27	0.12	2.11	0.03	1.57	0.01
14	2.33	0.18	2.29	0.15	1.80	0.24
15	2.11	0.04	2.13	0.01	1.56	0.00
16	1.83	0.32	1.87	0.27	1.51	0.05
17	2.25	0.10	2.17	0.03	1.75	0.19
18	2.39	0.24	2.04	0.10	1.33	0.23
19	2.12	0.03	2.01	0.13	1.29	0.27
20	1.95	0.20	2.00	0.14	1.49	0.07
21	2.18	0.03	2.19	0.05	1.58	0.02
22	1.71	0.44	2.28	0.14	1.47	0.09
23	1.89	0.26	2.04	0.10	1.45	0.11
24	1.93	0.22	2.15	0.01	1.53	0.03
25	2.27	0.12	2.28	0.14	1.69	0.14
26	2.08	0.07	2.22	0.08	1.57	0.01
27	2.07	0.08	2.23	0.09	1.75	0.19
28	2.23	0.08	2.39	0.25	1.41	0.15
29	1.92	0.23	2.19	0.05	1.62	0.06
30	2.23	0.08	2.46	0.32	1.49	0.07
31	2.46	0.31	1.81	0.35	1.76	0.20
32	2.17	0.02	1.93	0.21	1.75	0.19
33	2.12	0.03	2.46	0.32	1.51	0.05
Mean	2.15 ± 0.023 gm.		2.14 ± 0.023 gm.		1.56 ± 0.018 gm.	
σ	0.194 ± 0.017 gm.		0.195 ± 0.017 gm.		0.156 ± 0.013 gm.	
C.V.	9.02 ± 0.76 per cent		9.10 ± 0.091 per cent		10.0 ± 0.83 per cent	
P.E.	0.131 gm.		0.131 gm.		0.105 gm.	

* 33 cultures, 5 plants per culture; experiment continued 5 weeks; solution changed every 3½ days; results in dry weights per culture.

TABLE 3

Top weights, series A

NUMBER	R_5C_2		R_2C_5		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	2.04	0.35	1.83	0.12	1.64	0.34
2	1.90	0.21	1.85	0.14	1.31	0.01
3	1.63	0.06	1.54	0.17	1.36	0.05
4	1.52	0.17	1.62	0.09	1.29	0.01
5	1.74	0.05	1.40	0.31	1.45	0.15
6	1.67	0.02	1.98	0.27	1.34	0.04
7	1.68	0.01	1.58	0.13	1.14	0.16
8	1.88	0.19	1.58	0.13	1.29	0.01
9	1.81	0.12	1.80	0.09	1.09	0.21
10	1.67	0.02	1.61	0.10	1.09	0.21
11	1.80	0.11	1.71	0.00	1.25	0.05
12	1.73	0.04	1.74	0.03	1.17	0.13
13	1.73	0.04	1.71	0.00	1.35	0.05
14	1.92	0.23	1.81	0.10	1.59	0.29
15	1.75	0.06	1.72	0.01	1.35	0.04
16	1.35	0.34	1.51	0.20	1.27	0.04
17	1.76	0.07	1.76	0.05	1.46	0.16
18	1.84	0.16	1.62	0.09	1.12	0.18
19	1.63	0.06	1.65	0.06	1.08	0.22
20	1.53	0.16	1.54	0.17	1.25	0.05
21	1.67	0.02	1.70	0.01	1.31	0.01
22	1.37	0.32	1.78	0.07	1.25	0.05
23	1.50	0.19	1.61	0.10	1.19	0.11
24	1.58	0.11	1.71	0.00	1.27	0.03
25	1.71	0.02	1.86	0.15	1.40	0.10
26	1.62	0.07	1.79	0.08	1.27	0.03
27	1.66	0.03	1.81	0.10	1.44	0.14
28	1.78	0.09	1.90	0.19	1.16	0.14
29	1.48	0.21	1.72	0.01	1.30	0.00
30	1.87	0.18	1.87	0.16	1.27	0.03
31	1.48	0.21	1.48	0.23	1.48	0.18
32	1.55	0.14	1.55	0.16	1.46	0.16
33	1.98	0.31	1.98	0.27	1.33	0.03
Mean	1.69 ± 0.018 gm.		1.71 ± 0.017 gm.		1.30 ± 0.016 gm.	
σ	0.153 ± 0.013 gm.		0.14 ± 0.012 gm.		0.135 ± 0.011 gm.	
C.V.	9.05 ± 0.76 per cent		8.24 ± 0.69 per cent		9.66 ± 0.80 per cent	
P.E.	0.10		0.094		0.090	

DISCUSSION OF DATA

It is stated in the law of errors that when a number of variants are considered, large deviations from the mean will occur relatively less frequently than small ones; moreover, when a sufficiently large number of such variants

are taken, deviations will fall equally on either side of the mean. This is true, of course, only under ideal conditions where chance is not interfered with in any way. One can plot such data, frequency against measurement, and obtain the so-called frequency curve, the apex of a normal curve representing the true mean and departures from that mean falling equally on either side. In the case of a small number of variants, it is not likely that perfect symmetry would be obtained, since the law of chance would not have fair play, and more experimental values might fall on one side of the true mean than on the other. This would give the so-called "skew," or asymmetric curve. Such unbalanced distribution is not peculiar to few variants. Indeed, it is a common arrangement of biological data even where many variants are considered. In the data given in this paper, the deviation of individual determinations from the mean is relatively small, i.e., it never exceeds the mean in magnitude, while the distribution of such determinations about the arithmetic mean is in all cases such as to permit the construction of a symmetrical curve (fig. 5 to 10).

In case the extent of one's experimental data falls short of the ideal for the symmetrical curve (the usual condition), and yet is sufficiently extensive to give an approximation of symmetry, the normal curve can be calculated, and thus the experimental can be related to theoretical values. It is obvious that in such a comparison, the arithmetic mean may not coincide with the theoretical as indicated by the apex of the calculated curve; if there is a divergence, it must be assumed that the theoretical mean is the true one, since the law of probability demands symmetry. It becomes entirely possible, then, that one may fall into error in comparing different series of determinations through their arithmetic means alone, even although a relatively large series may be considered, since these may neither indicate the true mean nor show the influence of variability. The chances for misleading conclusions become much greater, however, when few determinations are averaged, particularly when stress is laid upon slight differences, for here, as will be shown later, the arithmetic mean may vary greatly from the more nearly true mean of a large series. In the data herein presented, the variatns considered, when grouped into classes give approximation of symmetry to the plotted frequency polygon, and from these, normal curves have been calculated which show graphically the extent of variability. Probable error,[4] standard deviation, and the coefficient of variability, have been determined for plants grown in

[4] The probable error of a single determination is given as that value lying on either side of the mean within which fifty per cent of the determinations of the series should lie. To find the probable error of a number of variants, use is made of the standard deviation, which is expressed as $\sigma = \frac{\Sigma d^2}{N}$, where Σd^2 is the sum of the squared deviations, N the number of variants, and σ the standard deviation. The probable error, PE, is then found by the formula $P.E. = 0.6745 \frac{\sigma}{\sqrt{N-1}}$, where 0.6745 is a constant. [See Davenport (3) and Merriam (6).]

each of the three solutions, and comparisons made in the light of these limiting factors. Assuming that the mean of any one solution series is accurate within its probable error,[5] we have a basis for judging the reliance which can be placed upon the means of duplicate cultures, or of any number which may be chosen at random from the series in question.

Series A

In this series, the culture (5 plants) rather than the individual plant is taken as a unit, thus following the common practice. Later, the variability shown by individual plants will be treated separately. The experimental data are given in tables 2 and 3, and are self-explanatory.

In Shive's original results, the values obtained for the means of two cultures are given as follows:

	R_5C_2	R_2C_5	R_1C_1
Dry weight, gm.	0.5704	0.4842	0.4104
With R_1C_1 as unity	1.39	1.18	1.00

Referring to table 2, however, we find the means for 33 cultures of R_5C_2 and R_2C_5 practically coincide. This is graphically shown in figures 1 and 2 where the corresponding calculated curves are compared. The curves show the existence of considerable variability for the cultures in question, which has resulted in an overlapping of data; in the case of the first two solutions being practically complete, and with R_1C_1 relatively slight. Even under the accepted conditions of control, the range due to variation is seen to be considerable and offsets completely any apparent difference between arithmetic means. It is evident that were we comparing means of duplicates, our conclusions might have been otherwise, since they would be influenced by the position occupied by the cultures represented within the area of the curve in question. A glance at the figures will show at once that such chance distribution might give results comparable to those of Shive, to those herein presented, or different from either in that R_2C_5 might be concluded the better solution. This point will be considered more in detail later.

In the case of R_1C_1, we have a solution high in magnesium sulfate and relatively low in nitrate and phosphate salts. *A priori*, one would expect a yield lower than that given by the other two solutions, conditions which are realized in the analysis of the results. The mean of 33 cultures is here 1.56 gm. as

[5] The mean of a number of variants cannot have a fixed value; the very fact of measurement introduces error. In biological data there is not only the error of measurement which may be relatively small, but errors as well due to an innate capacity to vary on the part of the organism. One must not only consider the mean in his calculations, but the probable error as well, since this, in the last analysis, fixes its accuracy. See Wood (14), Wood and Stratton (15), Merriam (6), Davenport (3) and texts on the law of probability, for discussion.

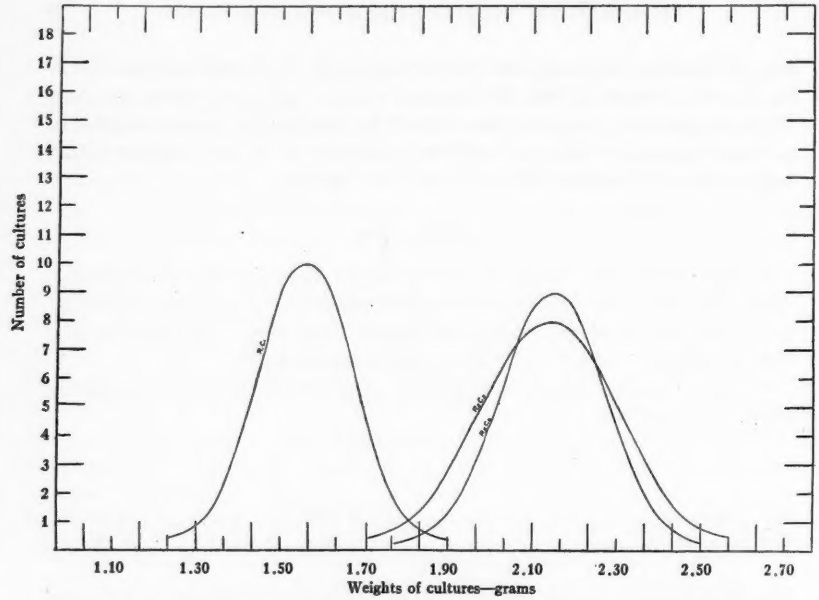

FIG. 1. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES A

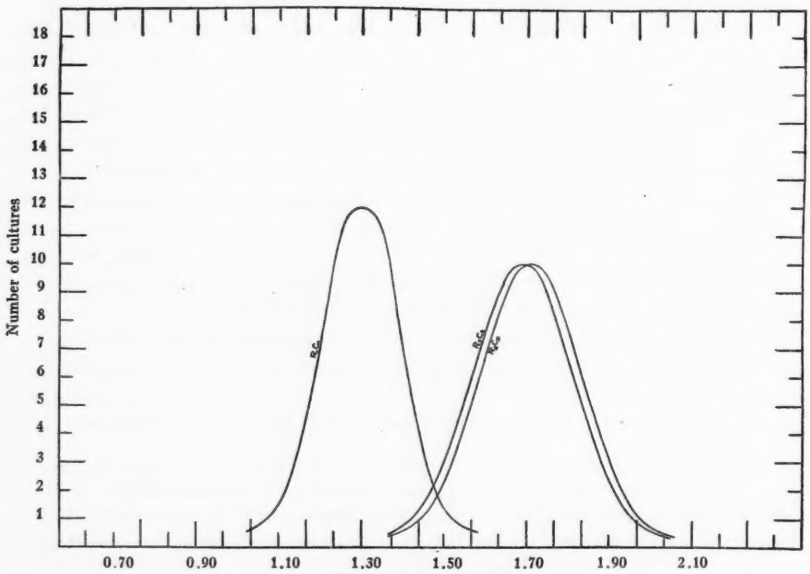

FIG. 2. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES A

compared with 2.15 gm. for R_5C_2, a difference of 0.59 gm., or about 27 per cent in terms of the latter solution. This difference is quite marked, and one might be justified in attaching significance to it. Referring to the curves in figures 1 and 2, however, it is seen that the overlapping, although slight, is such as to raise the question of its relative importance. How can one be certain that small numbers of cultures chosen at random from the series might not lie within this overlapping area? Since the ratio of this area to the area of the curves compared is small, it would be supposed that the chances for such an event occurring would be correspondingly slight. It is possible, however, to determine such odds when the probable error of difference between means is known.[6] Thus, in this case, for example, we have the means of these two solutions as 2.15 ± 0.023 gm. and 1.56 ± 0.018 gm., respectively, and by the formula given, we find this difference of 0.59 gm. has a probable error of 0.029 gm.

TABLE 4

Table of odds—Differences in both directions

DIFFERENCE FROM THE MEAN IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERECE OCCURRING UNDER UNIFORM CONDITIONS
1.00	1.41	1 to 1
1.25	1.76	3 to 2
1.44	2.03	2 to 1
1.71	2.41	3 to 1
1.90	2.68	4 to 1
2.00	2.83	9 to 2
2.05	2.87	5 to 1
2.50	3.53	10 to 1
2.93	4.13	20 to 1
3.00	4.24	22 to 1
3.20	4.51	30 to 1
4.00	5.66	140 to 1
4.90	6.93	1000 to 1
5.00	7.07	1350 to 1

Thus the actual difference between means is about 19 times its probable error. The significance of this value can be ascertained by reference to a table of odds, such as those included here under tables 4 and 5 which have been taken from Wood (14). In this case, the difference lies in one direction only, i.e., the effect of such a solution as R_1C_1 can be considered as varying in but one direction from R_5C_2, and so table 5 must be utilized. Taking the value 19 to the second column of this table, the corresponding odds in the third column, in favor of this difference being due to something other than normal variation, would be seen to be enormous were they computed to so high a figure. Cer-

[6] The probable error of difference is larger than that of either probable error alone, and is given by the formula: $A_1 - A_2 \pm \sqrt{E_1^2 + E_2^2}$, where A_1 and A_2 are the means, and E_1 and E_2 their respective probable errors.

tainly here this overlapping need not be considered and real inferiority may be assigned to the solution.

The above conclusions are made on the basis of 33 cultures where chance has had somewhat of an opportunity to locate a true mean. What would be the result were one to consider duplicates? In decreasing the number of cultures, the most apparent fact is the increase in the probable error, which

TABLE 5

Table of odds—Difference in one direction only

DIFFERENCE FROM THE MEAN IN ONE DIRECTION ONLY IN TERMS OF PROBABLE ERROR	DIFFERENCE BETWEEN TWO RESULTS IN ONE DIRECTION ONLY IN TERMS OF THE PROBABLE ERROR OF EACH RESULT	ODDS AGAINST SUCH DIFFERENCES OCCURING UNDER NORMAL CONDITIONS
1.00	1.41	3 to 1
1.25	1.76	4 to 1
1.44	2.03	5 to 1
1.58	2.23	6 to 1
1.71	2.41	7 to 1
1.81	2.55	8 to 1
1.90	2.68	9 to 1
2.00	2.83	10 to 1
2.48	3.50	20 to 1
2.70	3.81	30 to 1
2.89	4.07	40 to 1
3.00	4.24	44 to 1
3.03	4.28	50 to 1
3.44	4.85	100 to 1
4.00	5.66	290 to 1
5.00	7.07	2700 to 1

TABLE 6

Increase in probable error of the mean, with the decrease in the number of cultures—Series A

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R_5C_2	R_2C_5	R_1C_1	R_5C_2	R_2C_5	R_1C_1
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
2	2.15–0.095	2.14–0.095	1.56–0.075	1.69–0.071	1.71–0.066	1.30–0.067
4	2.15–0.066	2.14–0.065	1.56–0.052	1.69–0.050	1.71–0.047	1.30–0.043
8	2.15–0.047	2.14–0.047	1.56–0.038	1.69–0.036	1.71–0.033	1.30–0.033
16	2.15–0.034	2.14–0.034	1.56–0.026	1.69–0.025	1.71–0.023	1.30–0.023
33	2.15–0.025	2.14–0.023	1.56–0.018	1.69–0.018	1.71–0.016	1.30–0.016

varies as the square root of the number of cultures averaged. This is shown in table 6, where probable errors have been calculated for various numbers of cultures up to the 33 making up the series. Here it is seen that where the probable error of the mean total weights of both R_5C_2 and R_2C_5 is 0.023 gm., it increases to 0.095 gm. for duplicate cultures within these two series. Should we select any two cultures at random from either R_5C_2 or R_2C_5, their average by our definition of probable error would have but an even chance of coming

within the limits 2.05–2.25 gm. When we undertake to compare means of such low degree of accuracy as is exhibited here, it is perfectly evident that the difference between them must be considerable before we would be justified in assigning even relative efficiency values to these solutions. In the case at hand, it is possible to determine just how great this difference must be. We have found that the probable error of difference between R_5C_2 and R_2C_5 becomes 0.134 gm. when the comparison is made through the means of duplicate cultures. To obtain an even chance that a difference is real, it must equal the probable error of difference in magnitude, or in this case 0.134 gm. For the security of a 10 to 1 chance (table 4), a difference between solutions must exist equal to 2.83 times the probable error of difference (column 2), or in this case, $2.83 \times 0.134 = 0.379$ gm. However, a 10 to 1 chance is not considered sufficient to give security of the degree desired in this sort of work. It is more customary to demand the reliance of 30 to 1 odds, and if this were desired, one would not be justified here in attaching significance to a difference less than 0.53 gm., or 24 per cent of the mean.

It was noted above that the inferiority of R_1C_1, as shown by the mean of the series, was most evident. Is the larger probable error when duplicates are compared, sufficient to offset this difference in arithmetic means? The probable error increases to 0.078 gm. for duplicates and when this solution is compared with duplicates of R_5C_2, we find a difference of 0.59 ± 0.13 gm. The ratio of difference to probable error becomes 4.54, which, when taken into the second column (table 5) gives chances approximately 30 to 1 that the difference is due to something other than normal variation.

COMPARISON OF TOP WEIGHTS

There is considerable divergence of opinion among investigators regarding the relative value of total dry, top dry, or green weight, as a criterion of solution efficiency. The two former are employed as criteria here and when the means of 33 cultures are compared, no real difference in relative results is apparent (tables 2 and 3). In the comparison of top weights for duplicate cultures, however, the difference between R_1C_1, and R_5C_2 becomes less pronounced than where total weights were compared, thus:

$$1.69 - 1.30 \pm \sqrt{80^2 \text{ mgm.} + 67^2 \text{ mgm.}} = 0.39 \pm 0.105 \text{ gm.},$$

$$\text{and } \frac{0.39}{0.105} = 3.71$$

This value gives less than a 20 to 1 chance that the difference is significant. This, again, illustrates the error of considering too few cultures. The striking disparity between the means of these two solutions indicates a real difference, but because of overlapping due to variation, and the low accuracy of means of duplicates, one cannot have a chance better than 20 to 1 that it is real, unless the probable error is decreased by averaging a greater number of cultures. Thus, for 33 cultures, the probable error of difference decreases to 0.026 gm., and shows without question the real inferiority of the solution.

TABLE 7

*Total weight, series B**

NUMBER	R_5C_2		R_2C_5		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	534	59	440	20	422	2
2	484	9	487	27	432	12
3	582	107	525	65	430	10
4	468	7	471	11	428	8
5	489	14	481	21	470	50
6	507	32	482	22	452	32
7	483	8	479	19	454	34
8	424	48	408	52	458	38
9	525	47	417	43	380	40
10	545	65	464	4	375	45
11	547	18	461	1	425	5
12	428	47	449	11	428	8
13	465	10	472	12	390	30
14	504	29	469	9	408	12
15	482	7	544	84	461	41
16	430	45	460	0	436	16
17	476	1	482	22	398	12
18	418	59	394	66	413	7
19	445	35	383	77	452	32
20	385	90	443	17	426	6
21	455	20	452	78	412	8
22	492	17	457	3	430	10
23	494	19	451	9	361	59
24	513	38	517	57	421	1
25	412	63	522	62	365	55
26	424	49	411	49	434	14
27	431	44	459	1	428	8
28	504	30	455	5	408	12
29	404	68	479	19	340	80
30	453	22	426	34	459	39
31	427	48	538	78	376	44
32	536	61	451	9	400	20
33	484	11	424	36	440	70
34	524	45	454	6	438	18
35	509	34	438	96	458	38
36	404	67	479	19	362	58
37	434	41	389	71	422	2
38	527	52	390	70	424	4
39	452	23	448	12	440	20
40	520	45	458	2	425	5
41	561	86	494	34	383	37
42	465	16	411	49	436	16
43	468	7	509	49	461	41

* 50 cultures, 6 plants per culture; experiment continued 23 days; solution changed every 3 days; results in dry weights per culture.

TABLE 7—*Continued*

NUMBER	R_5C_2		R_2C_5		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
44	510	51	504	44	313	107
45	505	29	477	17	483	63
46	445	38	460	0	500	80
47	425	51	433	27	416	4
48	445	37	455	5	369	51
49	489	17	498	38	423	3
50	510	35	440	20	445	25
Mean	475 ± 4.3 mgm.		460 ± 3.8 mgm.		420 ± 3.5 mgm.	
σ	44 ± 3.0 mgm.		40 ± 2.7 mgm.		37 ± 2.5 mgm.	
C.V.	9.30 ± 0.62 per cent		8.70 ± 0.58 per cent		8.81 ± 0.59 per cent	
P.E.	30 mgm.		27 mgm.		25 mgm.	

SERIES B

Total weight

In this series, attempt was made to duplicate as nearly as possible the methods and technique employed by Shive. The quart jars used in the preceding series were replaced by 250-cc. wide-mouth bottles; solutions were changed every 3, instead of every 3½ days, and the experiment was carried on for 23 days, instead of for 5 weeks. Six plants made up a culture and 50 cultures were set up rather than 33. The average temperature was lower than for series A (table 1), and this, together with the shortened growing period, resulted in lower yields. Other possible temperature influences will be discussed later.

Reference to figures 3 and 4 clearly shows the variation existing within each of the three solutions and the overlapping due to this variation. While the means for R_5C_2 and R_2C_5 do not approach each other so closely as is true in series A, the three curves considered together are more closely associated than in that series. The value of such curves is here demonstrated. Were one to consider separate means without taking variation into account, the conclusion might be reached that the three solutions showed real differences. However, where the data are plotted in the form of a curve and the variation shown graphically, one can see at a glance that the question of overlapping, as caused by variation, must be seriously considered.

Table 7 shows the means of the three solutions in this series to be 475 ± 4.3 mgm., 460 ± 3.8 mgm., and 420 ± 3.5 mgm., for R_5C_2, R_2C_5, and R_1C_1, respectively. In this case, an appreciable difference seems to exist between the means of R_5C_2 and R_2C_5, the relative difference, in fact, being approximately that noted by Shive. Clearly, if arithmetic means alone are compared, the latter solution must be assigned an efficiency value less than that of R_5C_2.

TABLE 8

*Top weights, series B**

NUMBER	R_5C_2		R_2C_6		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	359	36	286	14	291	1
2	343	20	309	9	309	19
3	409	86	357	43	297	7
4	328	5	320	20	299	10
5	380	57	333	33	320	29
6	344	21	323	23	311	21
7	332	10	322	22	313	23
8	283	60	281	19	307	17
9	352	29	286	14	251	39
10	350	27	330	30	256	34
11	313	10	307	7	288	2
12	290	33	294	6	282	8
13	316	7	311	11	262	28
14	332	11	305	5	282	8
15	319	4	349	49	311	21
16	300	23	313	13	305	15
17	332	11	315	15	277	13
18	272	51	265	35	283	7
19	300	23	257	43	321	31
20	272	51	294	6	292	2
21	317	6	306	48	277	13
22	349	26	321	21	305	15
23	347	24	300	0	253	37
24	343	20	338	38	295	5
25	286	37	316	16	263	27
26	293	32	282	18	304	14
27	305	18	298	2	302	12
28	332	11	300	100	279	11
29	263	60	324	18	235	55
30	282	41	289	17	320	29
31	306	17	359	53	269	21
32	350	27	309	3	284	6
33	330	7	282	24	218	28
34	355	32	310	4	257	33
35	342	19	294	12	318	28
36	278	45	308	2	247	43
37	302	21	255	51	316	26
38	354	31	269	37	297	7
39	312	11	305	1	309	19
40	345	22	318	12	299	9
41	384	61	329	23	247	43
42	314	9	263	53	297	7
43	322	1	339	33	321	31

* 50 cultures, 6 plants per culture; experiment continued 23 days; solutions changed every 3 days; results in dry weights per culture.

TABLE 8—*Continued*

NUMBER	R_5C_2		R_2C_6		R_1C_1	
	Weight	Deviation from mean	Weight	Deviation from mean	Weight	Deviation from mean
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
44	297	26	328	22	206	84
45	339	16	307	1	329	39
46	310	13	303	3	345	55
47	288	35	284	22	282	8
48	298	25	296	10	266	24
49	340	17	339	33	286	4
50	341	18	284	22	299	10
Mean	323 ± 3.0 mgm.		306 ± 2.3 mgm.		290 ± 2.9 mgm.	
σ	31 ± 2.1 mgm.		24 ± 1.6 mgm.		30 ± 2.01 mgm.	
C.V.	9.60 ± 0.64 per cent		7.84 ± 0.52 per cent		10.35 ± 0.69 per cent	
P.E.	20.8 mgm.		16.1 mgm.		20.1 mgm.	

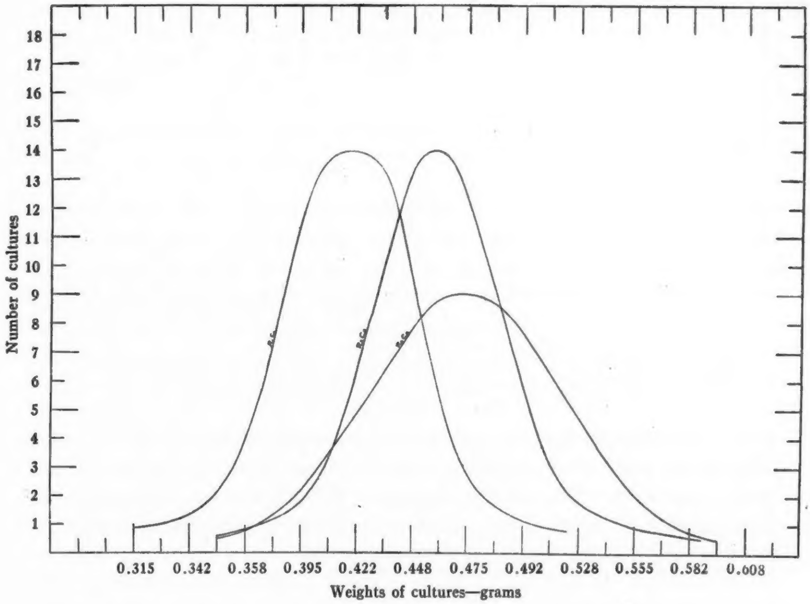

FIG. 3. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL CULTURES. SERIES B

In comparing R_5C_2 and R_2C_5, we find a difference of

$$475 - 460 \pm \sqrt{4.3^2 + 3.8^2},$$
$$\text{or, } 15 \pm 5.8 \text{ mgm.}$$

We do not know whether such a difference will always occur in the same direction, and hence, in the computing of odds, recourse must be had to table 4. The difference obtained is 2.58 times the probable error, a value which represents but a 4 to 1 chance that the discrepancy in means is due to a fundamental difference in solution and not to variability. To insure odds of

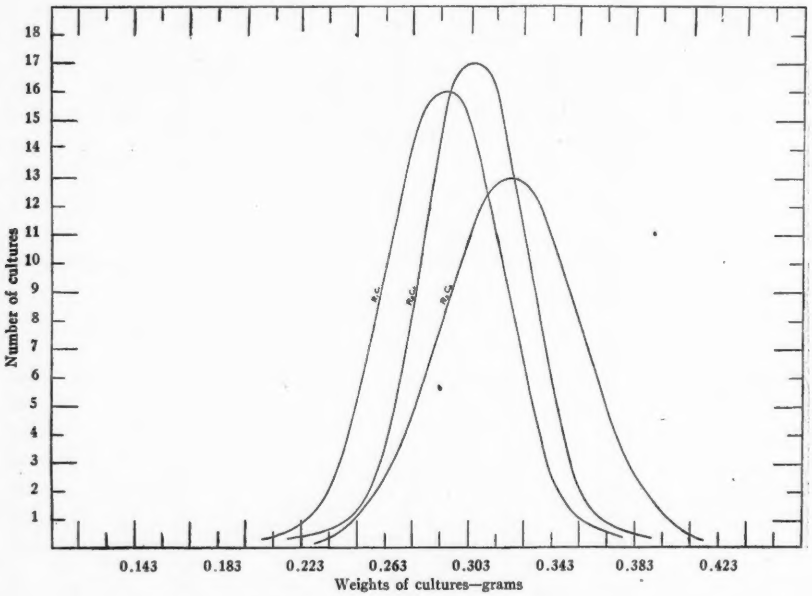

FIG. 4. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL CULTURES. SERIES B

30 to 1, the probable error of difference must be reduced to 3.3 mgm., or with the probable error as found a difference of at least 26 mgm. between the two means must exist. The disparity in means of R_5C_2 and R_1C_1, is again sufficiently great to warrant the previous conclusions regarding its inferiority, its value being 55 ± 5.7 mgm.

From table 9, we find the probable errors of duplicate cultures become 21 mgm., 19 mgm., and 18 mgm., for the three solutions in order. Once more, there is but a 1 to 1 chance that the mean of any two cultures will fall within the probable error of the mean of the series. In the case of R_5C_2 and R_2C_5, the difference in means is 15 ± 28 mgm. It is difficult indeed, to say that

this value means anything definite, for the probable error is of such magnitude as to annul completely the significance of difference. The question might arise that since there is an apparent difference between the solutions, and the probable error decreases with the number of cultures employed, all that is necessary is to decrease the probable error until such a point is reached as will give the security of chance desired. This might be done if one wishes to lay emphasis upon such small differences. It must be remembered, however, that the probable error decreases rapidly at first, then more and more slowly as the number of cultures is increased, and, to attain the accuracy desired, the number of cultures required might become prohibitive.

TABLE 9

Increase in the probable error of the mean, with the decrease in the number of cultures—Series B

NUMBER OF CULTURES	TOTAL WEIGHT			TOP WEIGHT		
	R_5C_2	R_2C_5	R_1C_1	R_5C_2	R_2C_5	R_1C_1
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	475±21.0	460±19.0	420±18.0	323±15.0	306±11.4	290±14.2
4	475±15.0	460±14.0	420±13.0	323±11.0	306±8.0	290±10.0
8	475±11.0	460± 9.8	420± 9.0	323± 7.4	306±5.8	290± 7.2
16	475± 7.3	460± 7.0	420± 6.2	323± 5.2	306±4.0	290± 5.0
32	475± 5.3	460± 4.8	420± 4.4	323± 3.6	306±2.8	290± 3.5
50	475± 4.3	460± 3.9	420± 3.6	323± 3.0	306±2.2	290± 2.8

THE RELATION OF THE MEAN OF DUPLICATE CULTURES CHOSEN INDISCRIMINATELY, TO THE MEAN OF THE SERIES IN WHICH THEY LIE

It has been shown above that odds in favor of the difference between two means being significant, decrease with a decrease in the number of samples averaged. Thus, for duplicate cultures, greater differences between the means of two pairs must be apparent than would be true for larger numbers. As stated in the introduction, the worker who employs duplicate cultures for comparative purposes assumes that no matter how many sets of duplicates he might use, the means would not vary enough from each other to vitiate his conclusions. The error of this assumption is most evident from the data in table 10. Here we have the mean of 33 cultures from series A as a check, and can select any number of pairs desired to see whether they approach this more accurate mean within reasonable limits. Sixteen pairs of cultures were selected by chance from the data for R_5C_2 of this series. Here, in a single solution and under a uniform set of conditions for all cultures, the means of these pairs so chosen range from 2.55 gm. to 1.82 gm., or 34 per cent of the mean of the series, 2.15 gm. Had we based our comparisons upon one of these random means and upon another chosen similarly from solution R_2C_5, it is seen that the conclusions reached would have depended entirely upon the mean which chance happened to throw in our way. It might be stated

however, that, when sampling at "random," the chances of getting a pair of values representing a large deviation from the true mean are not so great as they would be for a small deviation. In this case, we may take the probable error of two cultures, which has already been calculated for solution R_6C_2, i.e., 0.095 gm. Any haphazard selection, then, of the means of a pair of weights, such as given in the table, would have but a 1 to 1 chance of coming within the limits of 2.15 ± 0.095 gm. When we group these selected means in table 10 according to the relative size of their errors, we actually find that seven are found within such limits and nine outside, which is a rough approximation of the theoretical odds. The fact is here apparent in this experiment that one time out of two, we shall fall short of the true mean by at least one-tenth of a gram. Thus one would hardly be justified in attaching significance to a difference in the second, third, or fourth decimal place.

TABLE 10

Variations shown in the means of duplicate cultures chosen at random from series A

SELECTION NUMBER	VARIATION	SELECTION NUMBER	VARIATION
	gm.		*gm.*
1	2.22	9	1.97
2	2.18	10	2.05
3	1.97	11	2.37
4	2.15	12	2.25
5	2.34	13	2.09
6	2.00	14	2.55
7	2.16	15	1.99
8	2.28	16	1.82
Mean			2.15

VARIABILITY AS SHOWN BY INDIVIDUAL PLANTS

Although, as previously noted, certain workers have employed cultures of one plant each as the experimental unit, the common practice has been to use several plants to the culture. Conforming to this custom, we have in the foregoing discussion considered the culture as the basis for statistical treatment. It is perfectly obvious, however, that such collective units do not express the true variability of the individual plants, since the very act of grouping tends to offset any extreme errors which might be exhibited by the individuals alone. The literature has very little to say on this point as applied to water cultures. Brenchley (2) and Stiles (11), determined the probable error of the mean of 10 one-plant cultures. So few plants, however, cannot disclose the full extent of individual variation.

A difference of opinion exists regarding the relative advantages of cultures made up of one or several plants. The first condition gives optimum conditions for growth as far as interference by other plants is concerned, but the advantages of an average of several plants is lost.

The individual plants of series A were weighed separately and will here be treated as statistical units. Such data have not been placed in tabular form; it has been considered advantageous to treat them directly in connection with

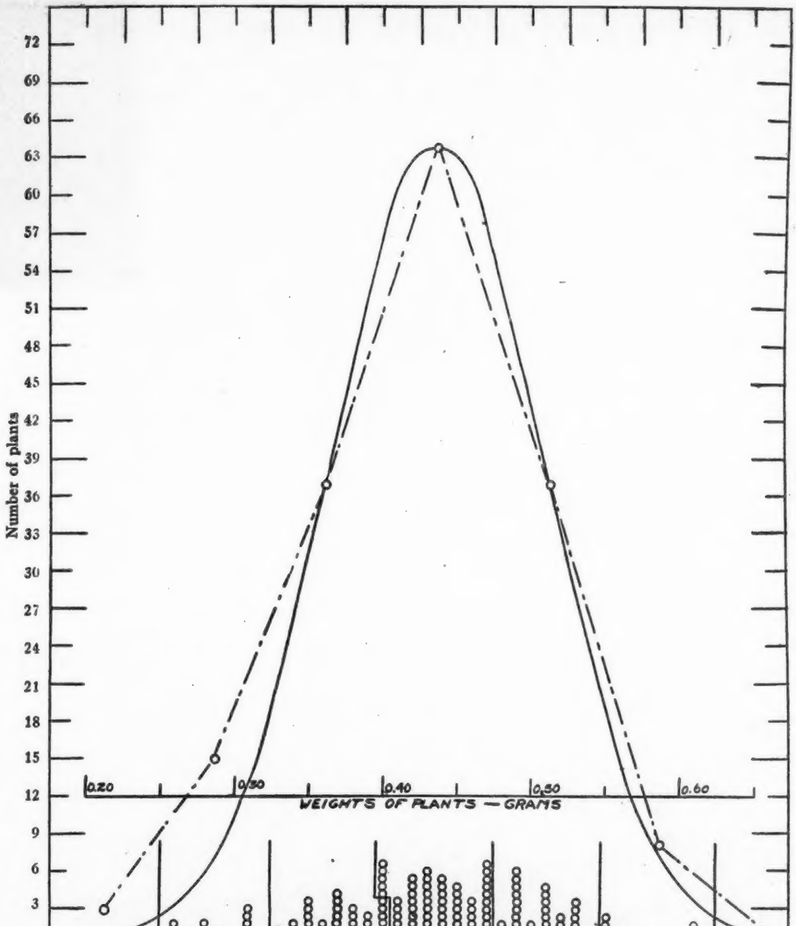

FIG. 5. SOLUTION R_5C_2; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

the constructed curves. Thus figures 5 to 10 show not only the polygons for experimental data (broken lines), but the calculated frequency curves and the original determinations from which the curves were constructed. These latter are shown as small circles at the base of the polygon making it possible to read

off directly the value of every determination of the 165 considered. In figure 5, for example, the separate determinations for R_5C_2 are so indicated. In plotting the experimental polygon, the weight values have been grouped

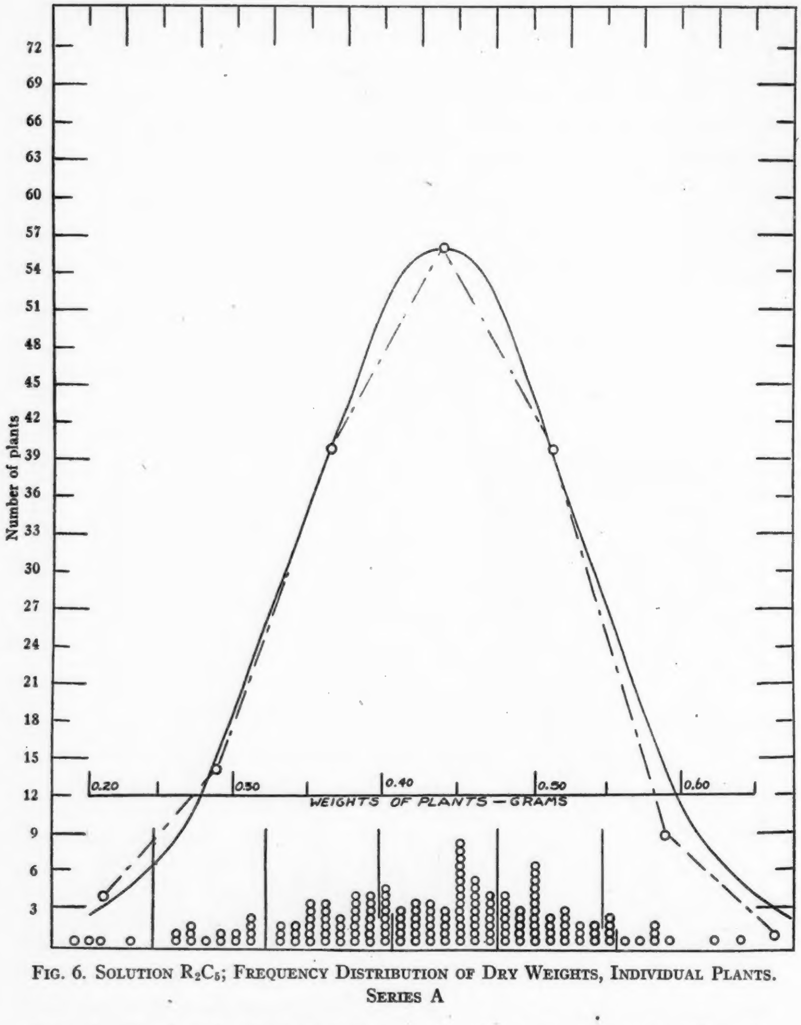

FIG. 6. SOLUTION R_2C_5; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

into classes, the distribution being such as to give an approximation of a normal curve. These classes are indicated by the heavy separating lines at the base of the figure, and the frequencies of determinations included in them are shown by the ordinate values. The various calculated values for the six

curves are given in table 11 where they may be compared. It is seen that here, as in the original series A the mean weights for solutions R_5C_2 and R_2C_5 are the same. In this case, the curve for R_2C_5 is the flatter of the two, show-

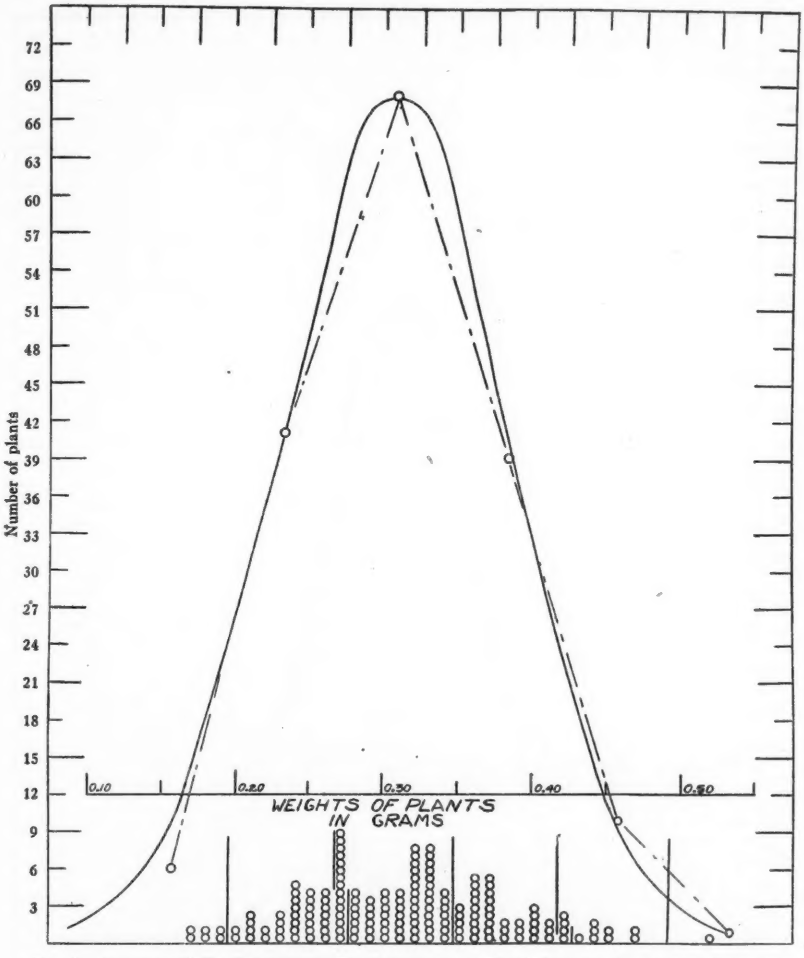

FIG. 7. SOLUTION R_1C_1; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

ing a wider range, i.e., a larger coefficient of variability. In fact, these coefficients of variability for both total weights and tops are shown to increase inversely as the values attributed to the solutions by Shive (7). Whether this fact is significant or not remains to be shown. It might indicate that

the real difference between these two solutions lies in the extent of variability which they exhibit rather than in their means. If this be true, R_2C_5 might be called less efficient than R_5C_2. However, we know practically nothing regard-

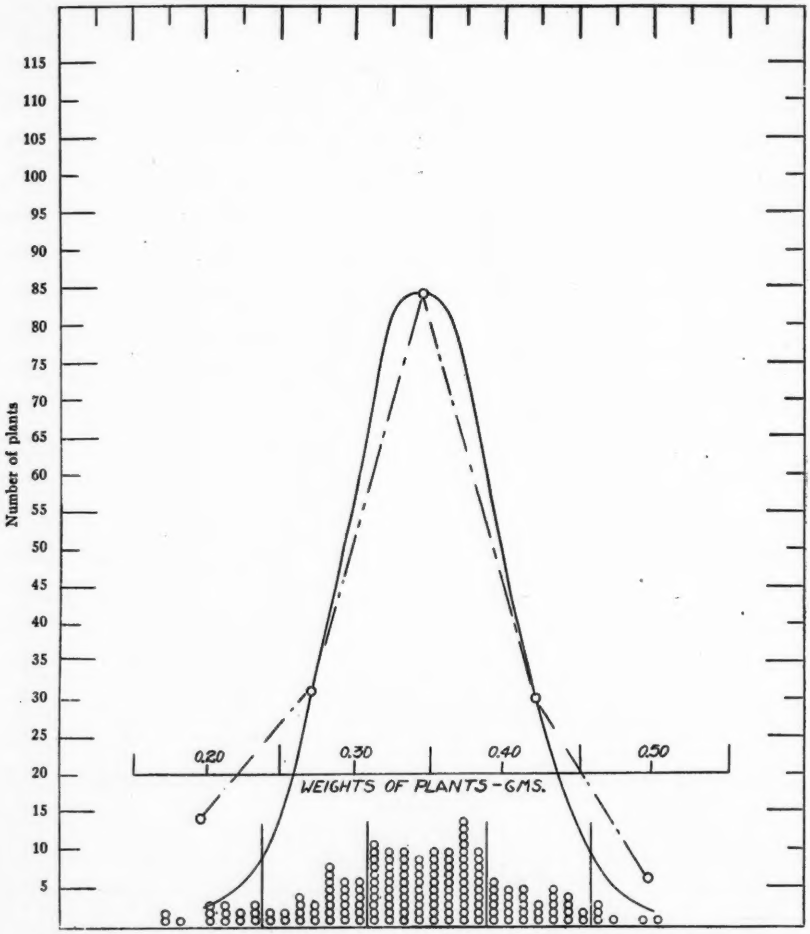

FIG. 8. SOLUTION R_5C_2; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

ing the effect of different culture solutions upon the variability of plants grown in them and certainly at the present time are not warranted in drawing distinctions on such a basis. Further study is required on this point.

In figures 11 and 12, this more extensive variation shows itself in an increased overlapping of the three curves as compared to that evident in figures 1 and 2. The difference between R_5C_2 and R_1C_1 remains significant, however, even

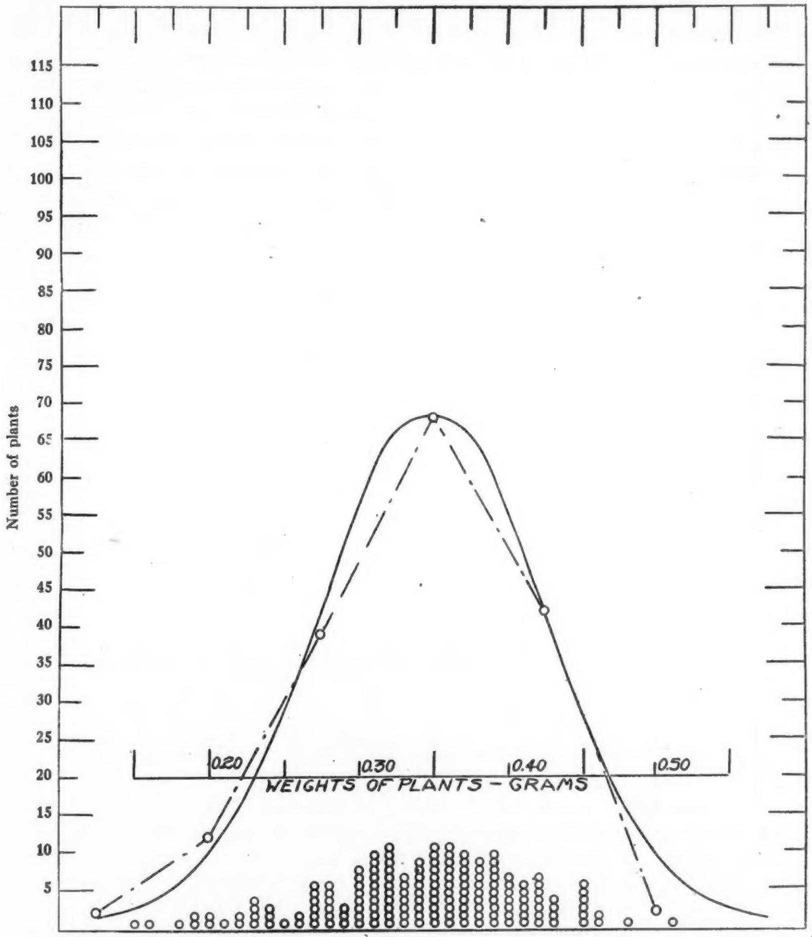

FIG. 9. SOLUTION R_2C_5; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

when compared through the probable error of 10 plants. This probable error becomes 11 mgm. for R_5C_2, and 12 mgm. for R_1C_1 and from these the probable error of difference on the basis of 10 plants is ± 16 mgm.

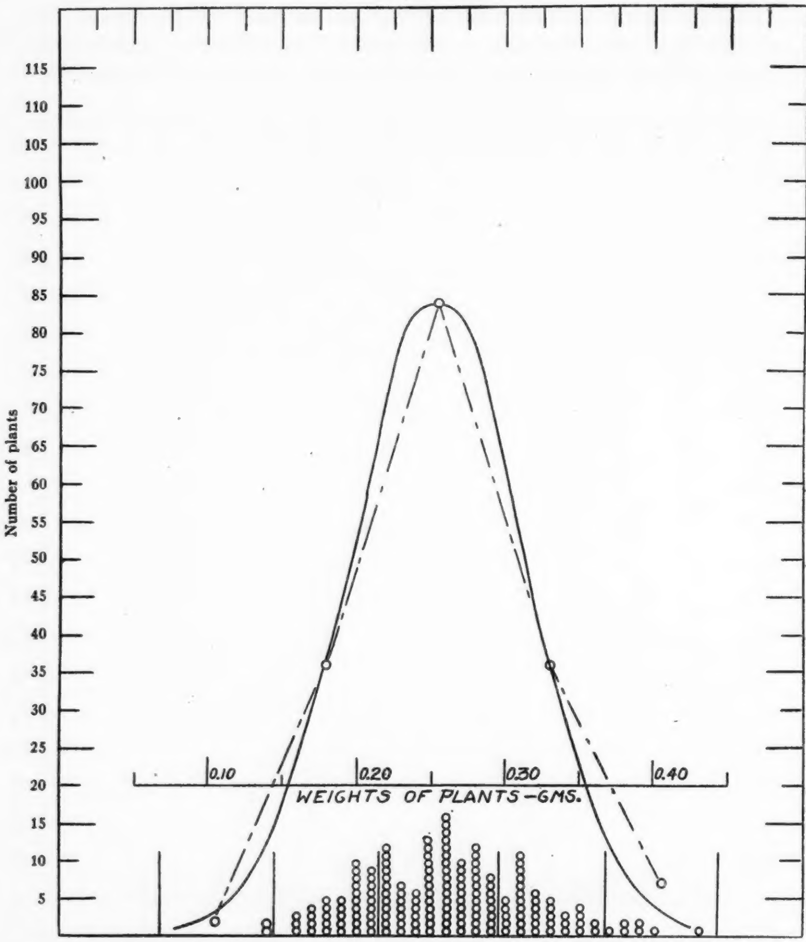

FIG. 10. SOLUTION R_1C_1; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

TABLE 11

Condensed table of values, individual plants—Series A

	WEIGHT OF COMPLETE PLANT			WEIGHT OF TOPS		
	R_5C_2	R_2C_5	R_1C_1	R_5C_2	R_2C_5	R_1C_1
Mean (mgm.)......	438±3.0	438±4.0	310±3.9	350±4.0	350±4.8	260±4.0
P.E. (mgm.).......	37	49	39	49	61	50
σ (mgm.)..........	55±2.0	73±2.7	58±2.2	73±2.7	91±2.4	75±2.7
C.V. (per cent).....	12.8±0.48	17.0±0.65	18.7±0.72	20.8±0.80	26.0±1.08	28.8±1.15

GENERAL DISCUSSION

It is seen from the results reported herein that plants grown in culture solutions exhibit considerable variability. Indeed, this is of such magnitude as to cast serious doubt upon the practice of drawing conclusions from the means of relatively few cultures. Whenever a worker concerns himself with material of so varying a nature as living organisms and seeks to compare the effect of differing environments, he must first of all ascertain the variation expressed by that material under the condition of the experiment. That such variations cannot be determined with any reasonable degree of accuracy with few variants is patent, and, because of this fact, the average of duplicates can have little meaning unless relatively large differences are being considered. Moreover, arithmetic means of even large numbers of determina-

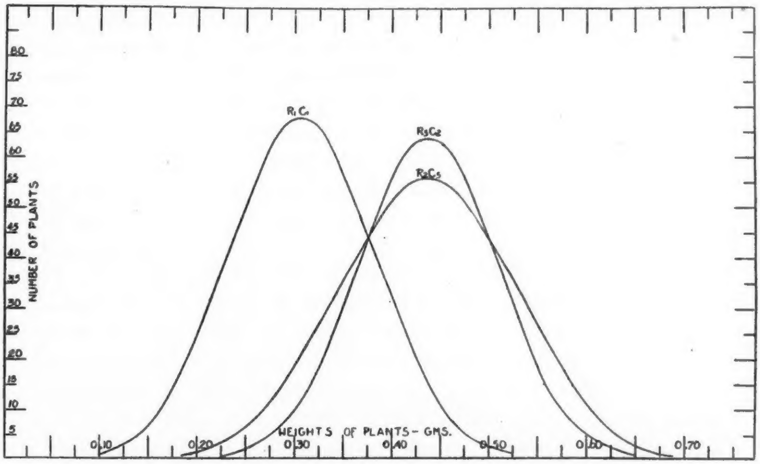

FIG. 11. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS, INDIVIDUAL PLANTS. SERIES A

tions of this sort cannot be considered as having fixed value; they are from the very nature of the case only accurate within certain limits, i.e., within their probable error. Until this error of probability is known, it is impossible to place absolute reliance upon the mean itself, no matter how many determinations are averaged. False values may be assigned them, and the conclusions formed be misleading.

In the culture solutions under discussion here, it is apparent that in neither series A, nor B, can a real difference between R_5C_2 and R_2C_5 be demonstrated, whereas had duplicate cultures been employed, the influence of variability upon their means could not have been determined, and solutions might have been given credit for differences due to variability. Thus in series B where

appreciable differences exist between the means of these two solutions, it would have been evidently misleading to have assigned definite values to the two arithmetic means. In Shive's (7) study, there are a number of solutions, such as R_2C_5, which lie outside the high or low-yield areas as mapped out on his triangles; in fact approximately half of the total number of solutions considered are found within such limits. It is to be wished that our limitations of time and facilities were not such as to make impossible the consideration of all such "medium" solutions, since it is felt that many of them, when interpreted statistically, would show no difference from those making up the high-yield area. Certainly the data reported here would indicate that the designation of a "best" solution, even for a given set of conditions is

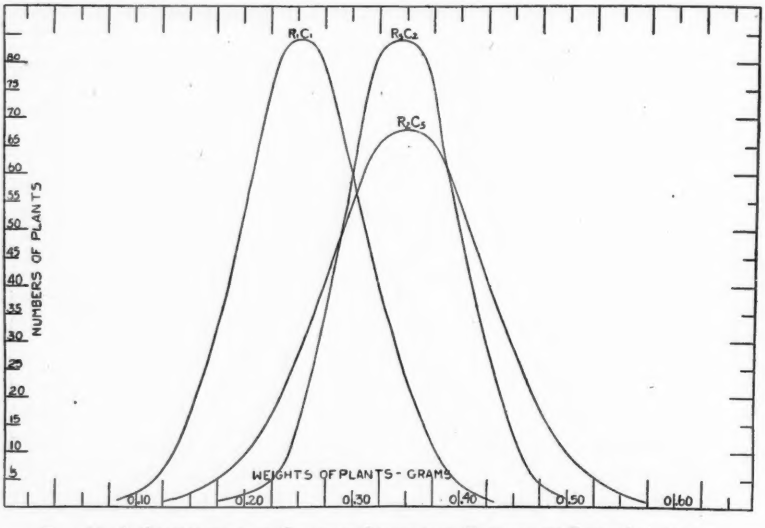

FIG. 12. A COMPARISON OF CULTURE SOLUTIONS; FREQUENCY DISTRIBUTION OF DRY WEIGHTS OF TOPS, INDIVIDUAL PLANTS. SERIES A

open to doubt. In fact it would seem that, as far as dry yield is concerned, the plant may respond equally well to a number of solutions having relatively wide limits in both total and partial concentrations of salts. In this connection, Stiles (11) noted that where total salt concentrations of 1, 1/5, 1/10, and 1/20 normal were compared, the probable error of the mean dry yield of 10 plants was of such magnitude as to nullify any differences shown by the means alone of the first three solutions.

In mapping out "high" and "low" yield areas, Tottingham (13), Shive (7, 8), and McCall (5) have set rather arbitrary limits. Thus in Shive's first paper (7), as well as in a later (8), the range between solutions giving highest and lowest values is divided into four parts, the upper one-fourth constituting

the high-yield area. In the first investigation, where yields of tops are compared, this high-yield area included but two solutions. From the data given herein, it is evident that, because of variability, such an arbitrary limit fails to give the true boundaries of the area in question. "High-yield areas" are really those made up of solutions giving yields which are not significantly different from those of the most efficient solutions. Thus, if we take R_5C_2 as such a standard, then those solutions would be excluded from the high-yield area whose mean yields give a difference from this standard large enough to be legitimately attributed to something other than normal variation. This would probably mean an enlargement of such areas, since undoubtedly certain "medium" solutions such as R_2C_5 would now have to be included.

McCall (5) in a study similar to Shive's (7) but in which sand was employed instead of water cultures, placed R_5C_2 in the low-yield area, while R_2C_7, a solution quite markedly different in salt proportions, gave the highest yield. Shive (8), on the other hand, in testing out the effect of the relation of moisture in sand cultures to salt balance, again found R_5C_2 the "best" solution. Wherein rests the explanation for these two widely divergent results? The first thought is of the conditions under which the two experiments were conducted. Shive (9), investigating the influence of sand on a nutrient solution, concluded that if the sand is carefully washed, no material alterations in the physiological properties of the nutrient solutions is to be expected, especially if they are frequently renewed. Both Shive and McCall employed washed sand. It is assumed that such conditions for these studies were not exactly the same, and yet they could hardly have varied sufficiently to be held responsible for the large difference noted. Does it not indicate rather that variability is the causal factor, and that in making comparisons on the basis of duplicate cultures, as has been done here, one is actually comparing fluctuating values? How is one to know whether the relative value assigned to R_5C_2 in Shive's work or in McCall's investigations, reveals the true efficiency of this solution? Obviously the only way we can be certain of comparative values in a study of this sort is to treat the solutions statistically and this can be done only by considering sufficient cultures to give a mean of fair degree of accuracy. This would, of course, preclude the consideration of large numbers of solutions differing slightly in salt proportions, but since it is apparent that small differences in partial salt concentration have little physiological significance, it would seem better to consider few such modifications, using relatively large numbers of replicates for each rather than many modifications employing duplicates only. Conclusions based on the former would have the value of being fairly reliable, while those based upon the latter might be entirely misleading.

In the comparison of culture solutions the importance of using seed of known parentage can hardly be overemphasized. Some varieties of wheat, for example, intercross readily under suitable conditions and the effect of this upon the variability of resulting plants may be marked. In this connection

it would be well worth while knowing whether variation is influenced significantly by different atmospheric environments, since it is usually only with difficulty that such conditions can be exactly duplicated. Again, we know nothing regarding the influence of different culture solutions upon variability. In such a series as Shive's, for example, where we find 36 modifications in partial molal concentration of the constituent salts, it would be of value to know whether any of these solutions increase or decrease the tendency of the plants growing in them to vary. It is expected that this point will be brought out in experiments now under way.

Comparison of many of the well known plant-culture solutions have been made from time to time, but it would seem that here a statistical comparison would be necessary in order to determine definitely their relative efficiency. Shive (7) for example, fixed such relative values for the conditions under which he conducted his experiment. It is doubtful, however, whether much reliance can be placed upon these assigned values, even under the limits which the author imposed, since an insufficient number of plants were employed to give a reliable mean. In fact, many of the studies heretofore made on culture solutions, while exceedingly important in their contribution to our knowledge of salt absorption, do not possess the convincing quality they would have if sufficient plants were employed to give a mean of known accuracy. Since plants do vary we must recognize the fact and allow for it in interpretations, otherwise conclusions may be faulty and future work built upon them prove to be wasted effort.

SUMMARY

The study herein reported concerns the variability shown by plants grown in water cultures and under the commonly accepted methods of control.

Three culture solutions were employed, selected from Shive's (7) 1.75-atmosphere series, and characterized by him as "best," "medium," and "poor" as regards growth efficiency. (The designations used by him were R_5C_2, R_2C_5, and R_1C_1, respectively.) They differed only in the partial molal concentration of constituent salts.

Two series were set up, series A in quart "Mason" jars, consisting of 33 replicate cultures of 5 plants each, and series B in 250-cc. bottles, made up of 50 replicate cultures of 6 plants each. Dry weights of complete plants and of tops alone were employed as criteria of variability.

The following general results were obtained:

1. The variation exhibited by plants of both series was of considerable magnitude, the range being approximately 20 per cent on either side of the mean for culture weights, and about 50 per cent when individual plants were compared. When results were plotted in the form of frequency curves and the curves compared, this variation was of such extent as to show considerable overlapping of data for the three solutions in question.

2. The arithmetic means of the "best" and "medium" solutions, series A, were practically the same, and the calculated frequency curves completely overlapped, thus showing no significant difference in the growth efficiency of these two solutions. In series B, an apparent difference was shown between the means of these two solutions, but the probable error was of such magnitude as to prevent assurance that this was due to anything other than normal variation. In both series the "poor" solution gave a mean significantly different from the other two solutions, thus attesting its real inferiority.

3. No difference was apparent between dry weights of the complete plant and tops alone as criteria of comparison.

4. A chance selection of duplicate cultures from series A gave means varying widely in value. Thus where the mean of the series was 2.15 gm., means of 16 pairs selected at random ranged from 1.82 to 2.55 gm.

It may be concluded that:

1. In comparing the relative growth efficiency of various culture solutions, the limits of accuracy of the means compared must be fixed, i.e., their probable errors must be ascertained, otherwise differences due to variation may be attributed to differences in the efficiency of the solution.

2. The mean of few cultures can have at best but low accuracy; it may vary greatly from the more nearly true mean of a larger series. Such a mean is valueless unless large differences are being compared.

3. There is no one "best" solution, at least under the conditions of control possible to maintain at present; undoubtedly the range of solutions favorable to plant growth is relatively extensive.

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A NEW CLASSIFICATION OF THE SOIL MOISTURE

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INTRODUCTION

The water in the soil is now generally divided into three forms: gravitational, capillary and hygroscopic. The gravitational water is defined as that which is in excess of the amount the soil can retain and which can, therefore, be drawn away by the force of gravity. The capillary water is that part retained in the capillary spaces of the soil and is capable of movement through capillary action. The hygroscopic moisture is the thin film on the surface of the soil particles and is not capable of movement through gravitational or capillary forces. This classification is based on the old idea that the soil is a static framework of solid particles varying in shapes and sizes over the surfaces of which the water spreads as a film and remaining unaffected by the soil and functions as a free liquid. This conception is well reflected by the attempts that have been made to measure the movement and average thickness of the films at definite moisture content, to extract this film of water, to calculate the specific surface of the soil from the size of its particles, etc.

With our present recognition of the colloidal and absorptive properties of the soil, however, we can no longer properly regard the soil as a simple, inactive mass of particles with no influence of its own upon the water. It is now generally admitted that colloidal material does exist in the soil derived both from the humus and clay fractions. This colloidal material is probably responsible for the major portion of the activity of the soil, exerts a controlling influence on the water relationships and makes these relationships between soil and water most intimate and complex.

The present classification of the soil moisture is probably serviceable for practical purposes, but is too general and empirical, and fails to reveal the true facts.

It is the purpose of this paper to present a new classification of the soil moisture which is founded upon a scientific basis, giving the true condition of the moisture in the soil and revealing the intimate and complex relationships that exist between soil and water. According to this new classification the soil water which has heretofore been classed as capillary does not exist in one form but in three different forms: free, capillary-adsorbed and combined.

Before this new classification is presented it will be profitable and advantageous to present first some data revealing the intimate and complex relationships that exist between soil and water and thus to show that the assumptions of the old classification are not correct and that a truer classification is needed.

THE COMPLEX RELATIONSHIPS EXISTING BETWEEN SOIL AND WATER

A few years ago we conceived the idea of trying to measure the concentration of the soil solution directly in the soil by means of the freezing-point method (4). This method has proved very successful and very unique for this purpose. We can now determine the concentration of the soil solution from any maximum to a very low moisture content directly in the soil. The results obtained by this method revealed the intimate and intricate relationships existing between soil and water most clearly and forcibly. It was shown, for instance, that in coarse-textured and uncolloidal soils such as quartz sand and extreme types of sand, the concentration of the soil solution increased

TABLE 1

Lowering of the freezing point of quartz sand at various moisture contents

MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CONSTANT K
per cent	°C.	
2	0.091	0.182
6	0.027	0.162
10	0.018	0.180
14	0.012	0.168
18	0.009	0.162

inversely proportionally to the moisture content, and could be expressed by the simple equation $MD = K$, where K is the resultant constant, M the percentage of moisture and D the observed depression of the freezing point. In the fine-textured and colloidal soils, on the other hand, the concentration of the soil solution increased in a geometric progression as the water content decreased in an arithmetic progression and could be mathematically expressed by the equation $D = AR^{n-1}$ where D is the freezing-point depression, A the first depression, R the ratio of any depression (except the first) to the preceding one, and N the number of the depression. Typical examples of these two sets of results are shown in tables 1 and 2.

It is readily seen, therefore, that in the case of the quartz sand the concentration increases in a direct ratio with the decrease of the moisture content, while in the case of the soil it increases at an abnormally greater rate than the moisture decreases.

The freezing-point method revealed the intimate and complex relationships that exist between soil and water still in another way. It was found that in all classes of soil, with the exception of sands, the magnitude of the freezing-

TABLE 2

Lowering of the freezing point of clay loam at various moisture contents

MOISTURE	OBSERVED LOWERING OF THE FREEZING POINT	CALCULATED LOWERING OF THE FREEZING POINT
per cent	*°C.*	
10.0	1.292	
12.5	0.612	0.956
15.0	0.377	0.453
17.5	0.252	0.279
20.0	0.162	0.186
22.5	0.112	0.120
25.0	0.082	0.083
27.5	0.050	0.060
30.0	0.037	0.037

TABLE 3

Effect of repeated freezing and thawing upon the lowering of the freezing point

MOISTURE	NUMBER OF TIMES FROZEN	LOWERING OF THE FREEZING POINT
	Sandy loam	
per cent		*°C.*
4	1	0.380
	2	0.340
	3	0.330
	Clay loam	
15	1	0.820
	2	0.530
	3	0.520
	4	0.515
	Silt loam	
16	1	0.430
	2	0.280
	3	0.220
	4	0.220
	Clay	
18	1	0.870
	2	0.600
	3	0.470
	4	0.465

point lowering at a low moisture content, decreased with successive freezing and thawing, and the magnitude of this diminution tended to increase with the fine texture and colloidal content of the soil. Typical examples of these results are shown in table 3.

These data show most strikingly and unmistakably, therefore, that the process of repeated freezing and thawing tends to reduce the initial freezing-point lowering considerably.

For an explanation of these phenomena as well as those showing that the freezing-point depression increases at an abnormally greater rate than the moisture content decreases, the following hypotheses were offered:

(a) The soils have the ability to cause a certain amount of water to become unfree. This unfree water may be in the soil either as capillary-adsorbed or chemically combined or both. In either event this unfree water is not free or active to function as a solvent but is removed from the active liquid phase and also from the field of action as far as the freezing-point lowering is concerned. Thus, if a clay causes 15 per cent of water to become unfree, and at 39 per cent of moisture this clay gives a depression of 0.075°C. and at 22 per cent 0.987°, then in the first case there is 24 per cent of moisture free or active to dissolve the salts, while in the second case there is only 7 per cent of free or active water for the same purpose. The depression of the freezing-point lowering at the low moisture content, therefore, would be many times greater than at the high, than would be expected from the total percentage of water.

(b) The water which the soils cause to become unfree and thus removed from the field of action as far as the freezing-point lowering is concerned, is due to the colloids which the soils contain and to the capillarities of the soil. A portion of the unfree water exists in the colloids as physically adsorbed and loosely chemically combined (3) and another portion as unfree capillary water in the capillarities of the soil. Upon freezing the colloids are coagulated, the bonds uniting them with the water break and some of the unfree water becomes liberated. The capillarities of the soil are also destroyed by the process of freezing and the unfree capillary water also becomes liberated. This liberated and now free water from both sources goes to dilute the original soil solution and thereby decreases the original lowering of the freezing point.

The results yielded by the freezing-point method together with the hypotheses advanced to explain them and the conclusions derived therefrom were confirmed completely and in a most remarkable way by the dilatometer method (2). In brief, this method has shown absolutely: (a) that soils do cause water to become unfree, (b) that the process of repeated freezing and thawing liberates some of this unfree water and (c) that the moisture which has heretofore been classed as capillary does not exist all in one form but can be divided into three different forms: free, capillary-adsorbed and combined.

The principle of the dilatometer method is based upon the fact that water expands upon freezing. Knowing the coefficient of expansion of water upon freezing and also the total water content of a soil, then the amount of water that freezes and does not freeze can be easily calculated.

The general procedure of the method consists of adding a definite amount of soil and water or a soil of known moisture content into the dilatometer,

filling the empty space with ligroin and then causing the moisture to freeze at different degrees of supercooling.[1]

It was found by this method that part of the moisture of a wet soil freezes very readily at slightly below 0°C., another portion does not freeze until a temperature of −4°C. was reached, and a third portion does not freeze at any temperature—not even down to −78°. In many clays and clay loams as much as 40 per cent of the water added failed to freeze even at the extreme low temperature of −78°, while in quartz sand and sands all the water added froze readily at slightly below 0°.

NEW CLASSIFICATION OF THE SOIL MOISTURE

Most obviously, therefore, the moisture in the heavier classes of soil above the hygroscopic point does not all exist in one form. If it all existed in one form it certainly should have all frozen at one temperature and at the temperature of slightly below 0° as was the case of the moisture in the quartz sand and sands. Since it required different degrees of cooling to freeze, and some did not freeze at all, then there seems to be a strong proof that the moisture which has heretofore been classed as capillary exists in more than one form in all the agricultural soils with the exception of the plain sands and some coarse sandy loams containing no organic matter. On the basis of the dilatometer method it would appear reasonable and logical to consider that portion of the water which freezes readily at slightly below 0° to be different from that which freezes at −4° and −78°C., and this to be different from that which does not freeze even at the extreme lower temperature. Accordingly, therefore, the moisture of the soils should be classified into the following forms on the basis of the dilatometer method:

$$
\begin{array}{l}
\text{Gravitational} \\
\text{Free} \\
\text{Unfree}\left\{\begin{array}{l}\text{capillary-adsorbed} \\ \text{combined}\left\{\begin{array}{l}\text{water of solid solution or} \\ \text{water of hydration.}\end{array}\right.\end{array}\right.
\end{array}
$$

The gravitational water needs no explanation of course. The free water is that which freezes for the first time at the supercooling of −1.5°C. The capillary-adsorbed water is that which freezes finally at the supercooling of −4° and also in the temperature of −78° minus the free water. The combined water is that which does not freeze at all.

The temperature of −1.5° has been chosen for the free water first because the freezing point of the soil is not 0° but somewhat less than 0°, and second because the rate of freezing near 0° is very slow. The temperature of −4° is chosen for the capillary-adsorbed water, first because this is the greatest degree of supercooling that the soil will withstand without premature solidification,

[1] For detailed description of the method and procedure see (2).

and second because at this temperature nearly all the water that is freezable will freeze. This is shown by the fact that very little if any additional water freezes at $-78°$ which does not freeze at $-4°$.

It appears logical to call all the water which freezes near 0° free water because pure water in mass freezes at 0°. Water which does not freeze at this temperature must be different from free water. Since the physical condition of the soil presupposes that some of its water must exist around and in the interstices of its particles, and that this water probably has a lower vapor pressure corresponding to a lower freezing point, it appears reasonable to call this water capillary-adsorbed. Water on the other hand, which does not freeze at all even at the extreme low temperature of $-78°$ must also be different from the capillary-adsorbed water. Since it is known that certain solid materials contain water of hydration, solid solution of water, etc., it has seemed reasonable to call this water which does not freeze combined.

RELATIVE AMOUNTS OF THE DIFFERENT FORMS OF WATER

The ability of the dilatometer method to distinguish and classify the moisture within any soil into the various forms between the various types of soil is revealed in tables 4 and 5. In these tables are presented the results of a very comprehensive list of soils representing all classes and many types. In table 4 the relative amounts of the different forms of water are based only on the water added, while in table 5 they are based both on the water added and also on the hygroscopic moisture.

The data in these tables reveal many facts of great interest. They show first of all that water in the soils does exist in different forms and that the amount of these different forms varies tremendously in the various soils. In some soils only one or two forms predominate, while in others all three are about equally divided. In sands and fine sandy loams it is the free water that predominates, which amounts in many cases to about 95 per cent of the total water present; the other 5 per cent consists as a rule of combined water; capillary-adsorbed water is apparently not common in most of these classes of soil. In loams and silt loams practically the same conditions hold except that more water is present in the combined form. In the clay loams, humus loams and clays it is the combined water that predominates followed in order by capillary adsorbed and free water, respectively.

SIGNIFICANCE OF THE NEW CLASSIFICATION OF SOIL MOISTURE

The dilatometer method not only affords us a true and scientific basis for the classification of the soil moisture into its proper forms but also gives us a deeper and truer insight regarding the behavior and functions of the soil moisture and its bearing upon other phenomena in the soil, such as the movement of moisture, evaporation, wilting coefficient, physiologically unavailable

TABLE 4

Relative amounts of the different forms of water based on the amount of water added

NUMBER	SOILS	FREE WATER		CAPILLARY-ADSORBED WATER		COMBINED WATER
		cc.	*per cent*	*cc.*	*per cent*	*per cent*
1	Quartz sand	5.0	100.0	0	0	0
2	Coarse sand	4.80	96.0	0	0	4.0
3	Medium fine sand	4.70	94.0	0	0	6.0
4	Illinois medium fine sand	4.70	94.0	0	0	6.0
5	Wisconsin Plainfield fine sand	4.45	89.0	0	0	11.0
6	Fine sandy loam	4.20	84.0	0	0	16.0
7	California Yolo fine sandy loam	4.20	84.0	0.20	4.0	12.0
8	California Harford fine sandy loam	4.25	85.0	0.15	3.0	12.0
9	Kentucky LaCrosse sandy loam	4.00	80.0	0.35	7.0	13.0
10	Illinois White silt loam	4.15	83.0	0.25	5.0	12.0
11	Kentucky Miami silt loam	4.05	81.0	0.05	1.0	18.0
12	Holland loam	3.40	68.0	0.70	14.0	18.0
13	Illinois Brown silt loam	3.40	68.0	0.50	10.0	22.0
14	Wisconsin Colby silt loam	3.50	70.0	0.20	4.0	26.0
15	Illinois brown silt loam	3.25	65.0	0.65	13.0	22.0
16	Wisconsin Carrington silt loam	3.05	61.0	0.25	5.0	34.0
17	Kentucky Carrington loam	3.05	61.0	0.15	3.0	36.0
18	Heavy brown silt loam	2.70	54.0	0.55	11.0	35.0
19	Heavy dark brown silt loam	1.70	34.0	1.50	30.0	36.0
20	Heavy dark brown silt loam	1.85	31.0	1.20	24.0	39.0
21	Kentucky Marshall silt loam	2.25	45.0	1.05	21.0	34.0
22	California Romona clay loam	2.30	46.0	1.00	20.0	34.0
23	Illinois black clay loam	1.70	34.0	1.50	30.0	36.0
24	California Chino silty clay loam	1.00	20.0	1.75	35.0	45.0
25	Kentucky Carrington clay loam					
26	Wisconsin Superior clay	1.60	32.0	1.50	30.0	38.0
27	Clay	1.20	24.0	1.80	36.0	40.0
28	Minnesota Superior clay	0.80	16.0	2.45	49.0	35.0
29	Norfolk sand, Coffee County, Ala	4.50	90.0	0	0	10.0
30	Norfolk sand, Anne Arundel Co., Ind	4.70	94.0	0	0	6.0
31	Norfolk sand, Johnson County, N. C	4.65	93.0	0	0	7.0
32	Dekalb sandy loam, Blount County, Ala	4.50	90.0	0	0	10.0
33	Dekalb sandy loam, Etowah County, Ala	4.35	87.0	0	0	13.0
34	Dekalb sandy loam, Blount County, Ala	4.30	86.0	0	0	14.0
35	Norfolk sandy loam, Jones County, Ga	4.65	93.0	0	0	7.0
36	Norfolk sandy loam, Bamberg County, S. C.	4.70	94.0	0	0	6.0
37	Norfolk sandy loam, Milles County, Ga	4.50	90.0	0	0	10.0
38	Norfolk fine sandy loam, Wayne County, Miss	4.40	88.0	0	0	12.0
39	Norfolk fine sandy loam, Bamberg County, S. C	4.60	92.0	0	0	8.0
40	Norfolk fine sandy loam, Winston County, Miss	4.50	90.0	0	0	10.0

TABLE 4—*Concluded*

NUMBER	SOILS	FREE WATER		CAPILLARY-ADSORBED WATER		COMBINED WATER
		cc.	*per cent*	*cc.*	*per cent*	*per cent*
41	Vernon fine sandy loam, Archer County, Texas	3.55	71.0	0.55	11.0	18.0
42	Vernon fine sandy loam, Archer County, Texas	3.70	74.0	0.60	12.0	14.0
43	Vernon fine sandy loam, Taylor County, Texas	3.85	77.0	0.15	3.0	20.0
44	Hagerstown loam, Polk County, Ga	3.80	76.0	0.45	9.0	15.0
45	Hagerstown loam, Lawrence County, Ala.	3.55	71.0	0.35	7.0	22.0
46	Hagerstown loam, Madison County, Ala.	3.95	79.0	0.10	2.0	19.0
47	Carrington loam, Barnes County, N. D.	2.20	44.0	0.80	16.0	40.0
48	Carrington loam, Goodline County, Minn.	1.60	32.0	1.90	38.0	30.0
49	Carrington loam, Sioux County, Ia	3.20	64.0	0.60	12.0	24.0
50	Summit silt loam, Cass County, Mo	2.40	48.0	1.15	23.0	29.0
51	Summit silt loam, Barton County, Mo	3.20	64.0	0.60	12.0	24.0
52	Miami silt loam, Delaware County, Ind	3.60	72.0	0.60	12.0	16.0
53	Miami silt loam, Hendricks County, Ind.	3.75	75.0	0.65	13.0	12.0
54	Miami silt loam, Boone County, Ind					
55	Memphis silt loam, Wilkinson County, Miss	2.45	49.0	1.10	22.0	29.0
56	Memphis silt loam, Wilkinson County, Miss	4.05	81.0	0.25	5.0	14.0
57	Marshall silt loam, Sioux County, Ia	2.40	48.0	1.00	20.0	32.0
58	Marshall silt loam, Goodline County, Minn	1.85	37.0	1.70	34.0	29.0
59	Marshall silt loam, Nodaway County, Mo.	2.40	48.0	1.15	23.0	29.0
60	Kirkland silt loam, Payne County, Okla	2.95	59.0	1.00	20.0	21.0
61	Kirkland silt loam, Payne County, Okla	3.25	65.0	0.75	15.0	20.0
62	Vernon clay loam, Kay County, Okla	2.65	53.0	0.60	12.0	35.0
63	Vernon clay loam, Archer County, Texas.	3.70	74.0	0.30	6.0	20.0
64	Vernon clay loam, Roger Mills County, Okla	3.25	65.0	0.65	13.0	22.0
65	Lufkin clay, Columbia County, Ark	2.50	50.0	1.10	22.0	28.0
66	Lufkin clay, Caddo Parish, La	3.50	70.0	0.50	10.0	20.0
67	Lufkin clay, Winston County, Miss	1.00	20.0	2.65	53.0	27.0
68	Houston clay, Franklin County, Texas	0.75	15.0	2.25	45.0	40.0
69	Houston clay, Grayson County, Texas	1.85	37.0	1.80	36.0	27.0
70	Houston clay, Ellis County, Texas	2.70	54.0	1.00	20.0	26.0
71	Cecil clay, Troup County, Ga	1.65	33.0	1.35	27.0	40.0
72	Cecil clay, Jackson County, La	0.80	16.0	2.45	49.0	35.0
73	Cecil clay, Randolph County, N. C	1.55	31.0	1.50	30.0	39.0

TABLE 5

Relative amounts of the different forms of water, based on the amount of water added and also on the hygroscopic water

NUMBER	SOILS	FREE WATER		CAPILLARY-ADSORBED WATER		COMBINED WATER
		cc.	*per cent*	*cc.*	*per cent*	*per cent*
1	Quartz sand	5.0	100.00	0	0	0
2	Coarse sand	4.80	94.12	0	0	5.88
3	Medium fine sand	4.70	91.10	0	0	8.90
4	Illinois medium fine sand	4.70	91.10	0	0	8.90
5	Wisconsin Plainfield fine sand	4.45	85.81	0	0	14.19
6	Fine sandy loam	4.20	80.00	0	0	20.00
7	California Yolo fine sandy loam	4.20	79.56	0.20	3.78	16.66
8	California Harford fine sandy loam	4.25	78.70	0.15	2.77	18.53
9	Kentucky La Crosse sandy loam	4.00	75.19	0.35	6.57	18.24
10	Illinois white silt loam	4.15	78.16	0.25	4.71	17.13
11	Kentucky Miami silt loam	4.05	75.70	0.05	0.93	23.37
12	California Holland loam	3.40	63.09	0.70	12.99	23.92
13	Illinois brown silt loam	3.40	59.66	0.50	8.77	31.57
14	Wisconsin Colby silt loam	3.50	61.40	0.20	3.52	35.08
15	Illinois brown silt loam	3.25	57.01	0.50	8.77	34.22
16	Wisconsin Carrington silt loam	3.05	53.50	0.25	4.38	42.12
17	Kentucky Carrington loam	3.05	51.26	0.15	1.93	46.81
18	Heavy brown silt loam	2.70	46.55	0.55	9.48	43.97
19	Heavy dark brown silt loam	1.70	29.56	1.50	26.09	43.35
20	Heavy dark brown silt loam	1.85	31.10	1.20	20.17	48.73
21	Kentucky Marshall silt loam	2.25	39.48	1.05	18.42	41.10
22	California Romona clay loam	2.30	38.34	1.00	16.67	44.99
23	Illinois black clay loam	1.70	16.70	1.50	23.55	49.75
24	California Chino silty clay loam	1.00	15.80	1.75	27.65	56.55
25	Kentucky Carrington clay loam					
26	Wisconsin Superior clay	1.60	26.40	1.50	24.75	48.85
27	Clay	1.20	19.80	1.80	29.70	50.50
28	Minnesota Superior clay	0.80	12.61	2.28	36.00	51.36
29	Norfolk sand, Coffee County, Ala	4.50	89.23	0	0	10.77
30	Norfolk sand, Anne Arundel County, Ind.	4.70	93.26	0	0	6.74
31	Norfolk sand, Johnson County, N. C.	4.65	92.28	0	0	7.72
32	Dekalb sandy loam, Blount County, Ala.	4.50	88.24	0	0	11.76
33	Dekalb sandy loam, Etowah County, Ala.	4.35	85.30	0	0	14.70
34	Dekalb sandy loam, Blount County, Ala.	4.30	84.30	0	0	15.70
35	Norfolk sandy loam, Jones County, Ga.	4.65	91.54	0	0	8.46
36	Norfolk sandy loam, Bamberg County, S. C.	4.70	92.52	0	0	7.48
37	Norfolk sandy loam, Milles County, Ga.	4.50	88.60	0	0	11.40
38	Norfolk fine sandy loam, Wayne County, Miss.	4.40	84.94	0	0	15.06
39	Norfolk fine sandy loam, Bamberg County, S. C.	4.60	88.80	0	0	11.20
40	Norfolk fine sandy loam, Winston County, Miss.	4.50	86.88	0	0	13.12

TABLE 5—*Concluded*

NUMBER	SOILS	FREE WATER		CAPILLARY-ADSORBED WATER		COMBINED WATER
		cc.	*per cent*	*cc.*	*per cent*	*per cent*
41	Vernon fine sandy loam, Archer County, Texas	3.55	65.14	0.55	10.09	24.77
42	Vernon fine sandy loam, Archer County, Texas	3.70	67.90	0.60	11.01	21.09
43	Vernon fine sandy loam, Taylor County, Texas	3.85	70.64	0.15	2.11	27.25
44	Hagerstown loam, Polk County, Ga	3.80	70.00	0.45	8.20	21.80
45	Hagerstown loam, Lawrence County, Ala	3.55	65.40	0.35	6.44	28.16
46	Hagerstown loam, Madison County, Ala	3.95	72.75	0.10	1.84	25.41
47	Carrington loam, Barnes County, N. D	2.20	39.43	0.80	14.36	46.21
48	Carrington loam, Goodline County, Minn	1.60	28.68	1.90	34.05	37.27
49	Carrington loam, Sioux County, Ia	3.20	57.35	0.60	10.75	31.90
50	Summit silt loam, Cass County, Mo	2.40	43.17	1.15	20.68	36.18
51	Summit silt loam, Barton County, Mo	3.20	57.55	0.60	10.77	31.68
52	Miami silt loam, Delaware County, Ind	3.60	67.42	0.60	11.24	21.34
53	Miami silt loam, Hendricks County, Ind	3.75	70.22	0.65	12.17	17.61
54	Miami silt loam, Boone County, Ind					
55	Memphis silt loam, Wilkinson County, Miss	2.45	42.62	1.10	19.13	38.25
56	Memphis silt loam, Wilkinson County, Miss	4.05	70.43	0.25	4.35	25.22
57	Marshall silt loam, Sioux County, Ia	2.40	41.17	1.00	17.16	41.67
58	Marshall silt loam, Goodline County, Minn	1.85	31.73	1.70	29.16	39.11
59	Marshall silt loam, Nodaway County, Mo	2.40	41.17	1.15	19.73	39.10
60	Kirkland silt loam, Payne County, Okla	2.95	51.76	1.00	17.55	30.69
61	Kirkland silt loam, Payne County, Okla	3.25	57.02	0.75	13.16	29.82
62	Vernon clay loam, Kay County, Okla	2.65	44.85	0.60	10.15	45.00
63	Vernon clay loam, Archer County, Texas	3.70	62.60	0.30	5.07	32.33
64	Vernon clay loam, Roger Mills County, Okla	3.25	55.00	0.65	11.00	34.00
65	Lufkin clay, Columbia County, Ark	2.50	46.00	1.10	20.26	33.74
66	Lufkin clay, Caddo Parish, La	3.50	64.46	0.50	9.21	26.33
67	Lufkin clay, Winston County, Texas	1.00	18.42	2.65	48.80	32.78
68	Houston clay, Franklin County, Texas	0.75	12.50	2.25	37.50	50.00
69	Houston clay, Grayson County, Texas	1.85	30.83	1.80	30.00	39.17
70	Houston clay, Ellis County, Texas	2.70	4.50	1.00	16.67	38.33
71	Cecil clay, Troup County, Ga	1.65	29.68	1.35	24.28	46.04
72	Cecil clay, Jackson County, La	0.80	14.39	2.45	44.05	41.56
73	Cecil clay, Randolph County, N. C	1.55	27.88	1.50	26.98	45.14

water, etc. Indeed with a knowledge of this new classification we are enabled not only to understand but also to predict what to expect regarding these phenomena. Thus for instance, in regard to the movement of moisture it could be safely predicted that a large percentage of water in the fine-textured or colloidal soils is immovable as far as capillary movement is concerned. This conclusion would be self-evident and justifiable from the fact that a large amount of water is not free to freeze in these soils even at the extreme low temperature of $-78°C.$, and if it is not free to freeze at this low temperature it is certainly not free to move capillarily.

As regards the rate of evaporation, it could be predicted and expected that the rate would be different at the various moisture contents. The free water would possess one velocity of evaporation, the capillary-adsorbed another and the combined still another. The free water would possess the greatest velocity of evaporation, the combined the smallest and the capillary-adsorbed an intermediate.

The phenomenon of the wilting coefficient of soils becomes also more intelligible in the light of the dilatometer results. The consensus of opinion among soils men and plant physiologists is that the plants wilt even when there is still plenty of moisture in the soil, because the movement of the moisture to the roots of the plants is not sufficiently rapid to supply the water lost by transportation. This consensus of opinion is well expressed by Shull (8). He says:

> The wilting of plants at the wilting coefficient of the soil can not be due to lack of moisture in the soil nor to lack of a gradient of forces tending to move water toward the plant. The view is held, therefore, that the wilting at this critical soil-moisture content must be due to the increasing slowness of water movement from soil particle to soil particle and from these to the root hairs, the rate of movement falling below that necessary to maintain turgidity of the cells of the aerial parts, even under conditions of low transportation.

In the light of the dilatometer results, however, the plants wilt not because the soil moisture does not move at a sufficiently rapid rate but because it does not move at all. As has already been seen the capillary-adsorbed water freezes with great difficulty and the combined not at all. Now if the soil moisture is not free to freeze it is certainly not free to move capillarily.

The more correct reasons for the wilting of plants at the wilting coefficient of soils appears to be the following: (a) The moisture near the wilting coefficient is held by the soil with such force that the plants can not extract it. A large part of this water is probably not in the liquid state. (b) Near the wilting coefficient the concentration of the soil solution is comparatively high which would tend to influence the intake of water by the roots. At the moisture content where there is no more free water but only capillary-adsorbed and combined the concentrations of the soil solutions, as proved experimentally (4), is greater than that of the cell sap of the roots.

The new physical classification of the soil moisture on the basis of the dilatometer results enables us now to classify the soil moisture also on a true

and scientific physiological basis. The old physiological classification of the soil moisture consists of dividing the soil water broadly into available and unavailable. From a practical standpoint this classification is probably serviceable but it is too general, empirical and does not give a true, detailed and definite information regarding the availability and unavailability of the different forms of water. The new classification, however, which is suggested by the results of the dilatometer method and supported by physiological studies seems to meet all these requirements. This new physiological classification of the soil moisture is as follows:

$$\begin{array}{ll}
\text{Gravitational} & \{\text{unsuitable or superavailable} \\
\text{Free} & \{\text{readily available} \\
\text{Unfree} & \left\{\begin{array}{l} \text{capillary-adsorbed}\{\text{very slightly available} \\ \text{combined}\left\{\begin{array}{l}\text{water of hydration} \\ \text{water of solid solution}\end{array}\right\}\text{unavailable}\end{array}\right.
\end{array}$$

According to this classification it is only the free water or the water which freezes at the supercooling of about $-1.5°$C. that the plants can take up very readily. For this water the plant exerts very little, if any, force to utilize it because it exists in a free condition and is not held very rigidly by any outside forces. It is this water with which the plant makes its growth. After the plant uses up this free water it generally begins to wilt.

The capillary-adsorbed water, or the water which freezes at the supercooling of $-4°$ and in the temperature of $-78°$ is available to the plant only slightly and under certain conditions. For this water the plant has to exert force to obtain it, because it is held by the soil with considerable force. With this water the plant is probably not able to make growth but simply to sustain life.

The combined water, or the water which does not freeze at all, even at the extreme low temperature of $-78°$, is ordinarily not at all available to the plant. This water probably exists in the solid phase and is held by the soil with tremendous forces.

This new physiological classification of the soil moisture appears to be amply supported by the large amount of data obtained on the wilting of plants and by a direct comparison made between the wilting coefficient and the different forms of water. The work of Briggs and Shantz (5) shows that plants wilt when the total soil moisture is still high which is considerably above the combined water. A direct comparison made between the wilting coefficient of some of these soils employed by Briggs and Shantz and the dilatometer method results show that the percentage of moisture which fails to freeze at the supercooling of $-1.5°$C. is very closely the same as that at which plants begin to wilt, indicating that the wilting coefficient of soils is at the point where the free moisture ends and the capillary-adsorbed moisture begins. On the other hand, the extensive investigations of Alway (1) in which he allowed the plants to grow almost to maturity show that the plants are able to reduce

the soil moisture down to the hygroscopic coefficient. Now this hygroscopic coefficient seems to represent about the same degree of moisture as the combined water, indicating, therefore, that the capillary-adsorbed water is available to plants under certain conditions.

In view of the close agreement that appears to exist between the wilting coefficient and the unfree water as determined by the dilatometer method, it would seem logical and advisable, therefore, to determine this factor by the dilatometer method. The percentage of water that fails to freeze for the first time at the supercooling of $-1.5°C.$ can be taken to represent the upper limits of moisture content at which plants may begin to wilt, while the percentage of moisture which fails to freeze at $-4°C.$ can be taken to represent the lower limits at which plants are able at all to extract the moisture from the soil under the most favorable conditions.

The determination of the wilting coefficient of soils by means of the plant, besides being tedious and time consuming, is not accurate nor constant. The investigations of Caldwell (6) and of Shive and Livingston (7) show that the permanent wilting of the plant is a function of the intensity of atmospheric evaporation. By means of the dilatometer method on the other hand, the determination of the wilting coefficient is more definite and more comparable and, of course, infinitely easier, more convenient and rapid.

The new classification of the soil moisture, therefore, is founded on experimental and scientific principles and appears to classify the soil moisture into its actual forms and thus reveals its actual conditions, its intimate and complex relationships with the soils and its bearing upon many phenomena in the soil.

SUMMARY

The object of this paper is to present a new classification of the soil moisture which is founded on experimental and scientific principles and which appears to show the actual condition of the moisture in the soil.

This new classification is based upon the principle of the freezing of water. It is found that a portion of the soil water freezes very readily near 0°C., another portion freezes only when a temperature of $-4°$ is reached and a third portion does not freeze at all, even at the extreme low temperature of $-78°C.$

Obviously, the water in the soil above the hygroscopic moisture, can not all be in one form, i.e. capillary, because if it were all in one form all of it ought to freeze at one temperature and indeed near 0°. Since different portions of it freeze at different temperatures or not at all, then it must exist in different conditions.

On the basis of these experimental and scientific facts the soil moisture lends itself to the following classification:

Gravitational
Free

$$\text{Unfree}\begin{cases}\text{capillary-adsorbed}\\ \text{combined}\begin{cases}\text{water of solid solution}\\ \text{water of hydration}\end{cases}\end{cases}$$

The free water is that which freezes for the first time at the supercooling of -1.5°C., the capillary-adsorbed water is that which freezes finally at the supercooling of -4° and at the cooling of -78°, minus the free water. The combined water is that which does not freeze at all, even at the temperature of -78°.

It appears reasonable and logical to call all water freezing near 0° free water because pure water in mass freezes at 0°. Water which does not freeze at this temperature must be different from free water. Since the physical condition of the soil presupposes that some of its water must exist around and in the interstices of its particles, and that this water probably has a lower vapor pressure corresponding to a lower freezing point, it appears reasonable and logical to call this water capillary-adsorbed. Water on the other hand which does not freeze at all even at the extreme low temperature of -78°C. must also be different from the capillary-adsorbed. Since it is known that certain solid materials contain water of hydration, solid solution of water, etc., it has seemed reasonable to call this water which does not freeze combined water.

On the basis of this classification it was found that in some soils only one or two forms of water exist while in others all three forms exist but in different proportions.

The method that is capable of measuring the relative amounts of these various forms of water in the soil is the dilatometer method. The principle of this method is based upon the fact that water expands upon freezing. If the coefficient of expansion of water is known and also the total percentage of water in the soil, then the amount of water that freezes or fails to freeze can be readily ascertained.

The procedure of the method consists of adding a definite amount of soil and water or a soil of known moisture content, into the dilatometer, filling the empty space with ligroin and then causing the soil moisture to freeze. It is first supercooled to -1.5°C. and the free water allowed to freeze at this temperature. Then the contents of the dilatometer are thawed and frozen at the temperature of -15° for half and hour. Then they are thawed again and supercooled to -4° where the capillary-adsorbed water freezes. When the contents come in equilibrium with the temperature of -4°, they may be cooled to -78°C. and brought back again to the temperature of -4°. It is not always necessary, however, to cool the soil to -78° because practically all the water that is freezable will freeze at -4°.

It is found that repeated freezing and thawing causes some of the unfree water to become free. This unfree water which becomes free belongs entirely to the capillary-adsorbed water.

The new classification of the soil moisture gives a clearer and deeper insight of the actual condition of the soil moisture, its intimate and complex relationships with the soil and its bearing upon many phenomena in the soil such as the movement of moisture, evaporation, wilting coefficient of soils, availability and unavailability of moisture, etc.

In the light of the dilatometer results, together with those of physiological studies, the soil moisture can now be classified more definitely and scientifically, also on a physiological basis. This new physiological classification of the soil moisture is as follows:

Gravitational	superavailable		
Free	very available		
Unfree	capillary-adsorbed	only slightly available	
	combined	water of solid solution	unavailable
		water of hydration	

The dilatometer method is capable of determining also the wilting coefficient of soils, and this determination is more accurate, definite and comparable than that by means of the plant, and of course, infinitely more convenient, easier and rapid.

The old classification of the soil moisture is too empirical and general and does not tell the true story of the actual condition of the soil moisture. It is based on the old idea that the soil is a simple mass of solid particles over the surface of which the moisture spreads and remaining unaffected by the soil itself. The soil, however, is a very complex mass and its relationships with water are very intimate and intricate. This old classification, therefore, must sooner or later give way to a new one which is based upon actual facts, such as the new classification proposed in this paper.

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SULFUR AND SULFUR COMPOSTS IN RELATION TO PLANT NUTRITION

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A few years ago Lipman and co-workers (8) called attention to the possibility of rendering insoluble phosphates available as fertilizers by composting them with sulfur. Data from soil composts were presented which showed that sufficient acid might be produced by the biological oxidation of sulfur to produce soluble phosphates, after the general manner of the manufacture of acid-phosphate. Although the basis of the earlier composting experiments was soil of various types, it seems to have been generally recognized that a liberal supply of organic matter is favorable to the process.

Brown and Warner (3) have secured results like those of Lipman, using manure as the basis of their composts. They found that the addition of sulfur increased the solubility of phosphorus, as compared with untreated, fermented manure. Composting rock-phosphate together with manure and sulfur led to remarkable increases of available phosphorus, as measured by extraction with solution of ammonium citrate. Ames and Richmond (1), working with soil composts, and Shedd (12), with composts of soil and manure, have obtained similar favorable effects of sulfofication upon the availability of rock phosphate.

EXPERIMENTATION

A. Composts

The results reported herewith were obtained by applying Lipman's procedure to the composting of "floats," or finely ground rock-phosphate, with manure or soil. In general, we have followed Lipman's methods closely, so as to make our results comparable with his, but the composts have been handled on a larger scale.

Two types of soil were used.[2] One was Miami silt loam, manured at the rate of 40 tons per acre; the other was a garden soil, prepared by composting Miami silt loam with manure, leaf mold and sod. Duplicate portions of compost were made as follows:

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] G. D. Williams should receive credit for performing this phase of the investigation.

1. 480 gm. soil.
2. 420 gm. soil and 60 gm. rock-phosphate.
3. 460 gm. soil and 20 gm. sulfur.
4. 400 gm. soil and 20 gm. sulfur and 60 gm. rock-phosphate.

All of these materials were dried by exposure to the air, ground and passed through a 40-mesh sieve, in preparation for use. The well mixed composts were placed in Mason jars of 1 quart capacity. Water was then added to the extent of 50 per cent of the total water-holding capacity, as measured by Hilgard's method (6). Afterward, the jars were weighed, covered loosely and stored at room temperature. At regular intervals the composts were restored to original weights by adding water. After intervals of 4, 8, 12 and 32 weeks they were mixed thoroughly and samples equivalent to 50 gm. of the original air-dried composts were dried by exposure to the air and pulverized for analysis.

The methods of analysis were as follows:

Citrate-soluble P_2O_5. Five grams of sample were extracted with 100 cc. of neutral solution of ammonium citrate kept at 65°C. for 30 minutes, according to the official method of agricultural chemists (2). An aliquot of the extract equivalent to 0.5 gm. of sample was evaporated after adding HNO_3 and $Mg(NO_3)_2$, and ignited. Final determination was by the volumetric method.

Water-soluble P_2O_5. Ten grams of sample were shaken with 160 cc. of water, originally boiling, for 30 minutes. Aliquots were treated as in the preceding determination.

SO_3. Five grams of sample were shaken with 100 cc. of 1.0 per cent HCl for 7 hours. SO_3 was determined by precipitation with $BaCl_2$ in the usual manner.

Total acidity. Extracts prepared as for determining water-soluble P_2O_5 were boiled and titrated with 0.02 N NaOH, using phenolphthalein as indicator. The results have been made comparable with those of Lipman by expression as equivalents of 120 gm. of compost.

H^+ *concentration.* This was determined by the colorimetric method of Gillespie (4), with extracts prepared as for determining total acidity.

The data resulting from these analyses are presented in tables 1 to 3, inclusive. Inspection shows that the total acidity increased regularly in the presence of sulfur. The greatest acidity was reached where sulfur alone was added. In this case, however, at the end of 32 weeks the value was only about 11 per cent of that found by Lipman, McLean and Lint (table 2) after 15 weeks of composting. Actual acidity (H^+ concentration, or pH) had practically reached its maximum after 12 weeks of composting. Conversion of the pH value to its equivalent of NaOH gives a value of 30 cc. or less 0.02 N alkali. Comparison of this value with that of total acidity shows that the latter was largely due to acid salts, rather than free sulfuric acid, as suggested by Lipman, McLean and Lint (8, p. 515). Accumulation of sulfates was much greater in the garden soil than in the silt loam, but it was as extensive where sulfur was supplied alone as where rock-phosphate was also added. Water-soluble P_2O_5 also increased to a much greater extent in the garden soil than in the loam, where both sulfur and rock-phosphate were added. These results probably depend upon the relative supplies of organic matter available for

TABLE 1

Changes in acidity of composts

TIME INTERVAL	SILT LOAM				GARDEN SOIL			
	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added
	Actual acidity							
weeks	pH	pH	pH	pH	pH	pH	pH	pH
12		6.9	2.9	3.4	7.0	6.8	3.2	3.6
32	5.4	5.5	3.1	3.7	7.3	7.2	3.3	3.7
	Total acidity 0.02 *N* alkaline							
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
0	14.6	14.6	14.6	14.6	17.1	17.1	17.1	17.1
4	15.8	8.5	24.8	44.9	17.1	10.7	70.5	53.4
8	11.5	9.4	106.8	115.3	21.4	8.6	128.2	102.5
12	35.0	20.0	343.9	282.8	15.8	11.5	208.0	249.9
32	21.4	21.4	730.5	551.1	11.5	13.7	527.6	376.0

TABLE 2

Changes in percentage of dilute HCl-*soluble* SO_3 *of composts*

TIME INTERVAL	SILT LOAM		GARDEN SOIL	
	Sulfur added	Rock-phosphate and sulfur added	Sulfur added	Rock-phosphate and sulfur added
weeks	*per cent*	*per cent*	*per cent*	*per cent*
0	Trace	Trace	Trace	Trace
4	0.25	0.42	1.17	1.00
8	0.42	0.58	2.00	1.83
12	0.42	0.67	2.25	2.42
32	1.10	1.25	2.75	2.75

TABLE 3

Changes in percentage of soluble P_2O_5 *in composts*

TIME INTERVAL	SILT LOAM				GARDEN SOIL			
	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added	Control	Rock-phosphate added	Sulfur added	Rock-phosphate and sulfur added
	Water-soluble P_2O_5							
weeks	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
32	Trace	0.002	Trace	0.019	0.016	0.015	0.013	0.052
	Citrate-soluble P_2O_5							
0	0.06	0.34	0.06	0.34	0.09	0.32	0.09	0.32
4	0.06	0.34	0.07	0.33	0.10	0.34	0.11	0.35
8	0.06	0.30	0.05	0.29	0.12	0.36	0.11	0.33
12	0.04	0.30	0.05	0.35	0.11	0.34	0.10	0.32
32		0.27		0.38		0.28		0.42

bacteria in the composts. Citrate-soluble P_2O_5 remained unchanged, with the exception of an increase in the garden soil during the last period of the investigation. Had account been taken of the loss of dry matter by fermentation, this constituent must have shown a decrease on a percentage basis. It should be noted that in the similar work of Shedd (table 3) increase of citrate-soluble P_2O_5 in the complete compost was not detected until fermentation had proceeded for 7 months, and was first found to be extensive after 15 months, while in the tests of Lipman, McLean and Lint with greenhouse soil (8, table 2) the production of this constituent became very active between 3 and 4 months of fermentation. This difference may be due to differences of temperature and aeration.

A series of composts with manure only and with a mixture of manure and soil as basal materials also were prepared. Fresh horse manure was air-dried and mixed with the other compost materials. The composts were inoculated with a water extract of fresh manure and allowed to ferment for 15 weeks. Although the total acidity increased three to seven-fold where sulfur was added, the percentage of citrate-soluble P_2O_5 remained practically stationary. The duration of this experiment should be noted in comparison with that of experiments subsequently described.

A similar test of manure composts was conducted on a larger scale.[3] Fresh cow and horse manures were freed from coarse litter and mixed in the proportion of 2 to 1, by weight. Samples were taken immediately for bacterial counts on agar media and for chemical analysis. Six portions of 4540 gm. (10 lbs.) each were then placed in glazed stoneware jars of a capacity of 2 gallons each. Additions were incorporated as follows:

Jar 1. Nothing.
Jar 2. Nothing (eventually received 32 gm. $Ca(H_2PO_4)$ and 146.7 gm. $CaSO_4.2H_2O$.
Jar 3. Nothing (eventually received 65 gm. rock-phosphate and 21.4 gm. sulfur).
Jar 4. 150 gm. rock-phosphate and 50 gm. sulfur.
Jar 5. 150 gm. rock-phosphate.
Jar 6. 50 gm. sulfur.

The added materials had been sifted through a 100-mesh sieve. After covering with burlap bags, the contents of the jars were incubated at room temperature. At intervals of 32 and 87 days the composts were mixed and the control and those which had received additions were sampled and analyzed as at the beginning. It was impracticable to continue this experiment longer. Loss of organic matter by fermentation was determined by weighing the composts before and after sampling, thus rendering possible the computing of data from successive analyses to the common basis of original dry matter. Extracts were prepared by the use of water and ammonium-citrate solution successively, after the manner of Tottingham and Hoffmann (14), excepting

[3] J. A. Wolfram should receive credit for performing the laboratory and greenhouse work of this experiment.

the determination of citrate-soluble P_2O_5 at the last analysis, for which Lipman's method was followed. At the last analysis also in addition to titrating the usual water extract for acidity, a separate sample was extracted with hot water, following Lipman's method.

The data derived from this experiment are assembled in table 4. As shown in column 2 the loss of dry matter was greatest in the untreated manure. Naturally, the introduction of rock-phosphate reduced the percentage loss of dry matter. We have not retained the necessary data for computing the loss of organic matter for comparison with the control. The two composts just referred to continued to ferment actively in the second period of the investi-

TABLE 4

Composition of composts after various periods of fermentation based upon the original dry matter

COMPOST MATERIALS	RESIDUAL DRY MATTER		BACTERIAL COUNT PER GM.			TOTAL ACIDITY (0.1 N alk.)				WATER-SOLUBLE SO_3			WATER-SOLUBLE P_2O_5			CITRATE-SOLUBLE P_2O_5		
	32 days	87 days	Beginning	32 days	87 days	Beginning	32 days	87 days		Beginning	32 days	87 days	Beginning	32 days	87 days	Beginning	32 days	87 days*
	per cent	*per cent*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Manure.	71.2	58.5	206	214	158	20.5	11.6	9.7	19.7*	0.21	0.09	0.03	1.05	0.65	0.57	0.35	0.48	0.20
Manure, rock-phosphate and sulfur.	88.4	85.2	206	326	316	25.2	23.6	45.0	49.2	0.21	0.47	0.54	1.23	1.12	1.31	0.46	0.46	0.83
Manure and rock-phosphate.	80.0	70.7	206	400	247	24.0	11.2	10.4	14.8	0.20	0.07	0.04	1.28	0.82	0.43	0.46	0.58	0.21
Manure and sulfur.	91.0	89.2	206	356	366	32.2	28.6	40.2	39.6	0.40	0.36	0.54	1.13	1.11	0.99	0.37	0.21	0.92

* Lipman's method.

gation, while the losses of dry matter where sulfur was added were small during this period. The least loss occurred where sulfur was added alone. Where rock-phosphate was added alone the bacterial counts indicate decided stimulation of these organisms in the earlier period of the investigation, but the count falls in the second period. It will be noted that a somewhat smaller increase of bacteria in the composts containing sulfur was maintained throughout the experiment. These relations suggest important modifications of biological factors, dependent upon the kind of inorganic materials introduced into composting practice.

As regards total acidity, this factor had increased decidedly over 87 days where sulfur was present, but the effect was not apparent after 32 days. This indicates a relatively late predominance of the sulfur-oxidizing bacteria. The results of our method of extraction probably agree with those of Lipman's method as well as should be expected. It should be noted that the increase of acidity in these composts is not sufficient to promise much increase of availability of rock-phosphate also added. Excepting a high initial value in the sulfur-treated compost, the changes of sulfate parallel those of acidity.

Water-soluble P_2O_5 decreased appreciably in the manure and the compost with rock-phosphate, as fermentation progressed. This is in agreement with results previously reported (14). In the composts where sulfur was added the amount of water-soluble P_2O_5 remained practically unchanged. Citrate-soluble P_2O_5 showed significant variations only at the last analysis. It should be noted that the data there obtained are less than those for the corresponding water-soluble P_2O_5 in all cases. Compared among themselves the data of Lipman's method showed increased availability of P_2O_5 where sulfur was added with rock-phosphate. However, the available P_2O_5 in this case was about the same as where sulfur was composted alone. Similar relations obtain in the water-soluble P_2O_5 at the last analysis. The variations of citrate-soluble P_2O_5 are parallel to those of acidity, at the close of the fermentation period.

Application of the composts just described to greenhouse cultures of oats upon Miami silt loam and Plainfield sandy loam has confirmed the general indications of availability of P_2O_5 derived from the chemical analyses. Portions of each type of soil in the air-dried state, weighing 12.75 kgm. in the case of silt loam and 16.75 kgm. with sandy loam, were placed in boxes of galvanized iron 30 cm. square and 20 cm. deep. The inner surface of the boxes had been previously coated with paraffin. At this time, as previously indicated, additions of sulfur and rock-phosphate were incorporated with the residual manure of jar 3, and jar 2 received mono-calcium phosphate and calcium sulfate. These additions conveyed approximately the same proportions of P_2O_5 and sulfur as were involved in the other composts. Computed amounts of manure and composts, each equal to 100 gm. of the original fresh manure, were now tilled into the surface soil of the various boxes. A pedigreed strain of oats was then sown and the soils were sparingly and uniformly watered until the seedlings were 7 to 8 cm. high. The cultures were now reduced to 16 plants per box, uniformly distributed, and the water supply was controlled by weighing. At first the water plane was maintained at 30 per cent of the full water-holding capacity of the two soils. Two weeks later it was raised to 40 per cent and when signs of maturation appeared on the plants it was reduced. The cultures were rotated in periods of 2 or 3 days as to position upon the benches, for the purpose of counteracting effects of variations of temperature and illumination within the greenhouse. Plates 1 and 2 show the appearance of one set of the duplicate series of cultures conducted with each soil. These were taken after 15 weeks of growth and 2 weeks before harvesting. The plants

were crowded together as shown only for the purpose of photographing. When harvested the crops were dried at about 55°C. and exposed to the air at room temperatures for several days before weighing. Data of the yields

TABLE 5

Effects of composts on the yield of air-dried seed by oats

MANURIAL ADDITIONS	NOTHING	FERMENTED MANURE	FERMENTED MANURE, $CaSO_4$ AND $Ca(H_2PO_4)_2$	FERMENTED MANURE, SULFUR AND ROCK-PHOSPHATE	COMPOST OF MANURE, SULFUR AND ROCK-PHOSPHATE	COMPOST OF MANURE AND ROCK-PHOSPHATE	COMPOST OF MANURE AND SULFUR
On silt loam							
Culture 1 (gm.)....	7.4	9.3	11.7	11.2	12.3	9.4	10.0
Culture 2 (gm.)....	8.3	9.2	9.7	11.1	14.0	10.0	8.0
Average (gm.).....	7.8	9.3	10.7	11.2	13.2	9.7	9.0
Relative (per cent).		100	115	120	142	104	97
On sandy loam							
Culture 1 (gm.)....	5.8	7.3	5.3	8.2	9.3	6.6	11.5
Culture 2 (gm.)....	5.3	5.2	5.5	8.8	10.1	7.9	10.3
Average (gm.).....	5.6	6.3	5.4	8.5	9.7	7.3	10.9
Relative (per cent).		100	86	134	154	116	173

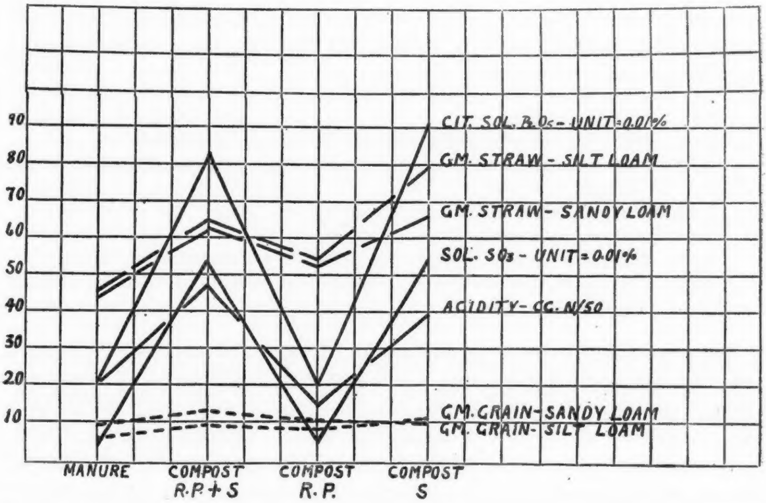

FIG. 1. COMPARISON OF YIELD (SOIL CULTURES) WITH COMPOSITION OF 12-WEEK COMPOSTS

are assembled in table 5. Inasmuch as the predominant effects of variations in the supply of P_2O_5 appear in seed production, we have omitted yields of straw from the table. Figure 1 gives a comparison of yields of both grain and straw with acidity, water-soluble SO_3 and citrate-soluble P_2O_5 of the composts.

The general agreement between high yields of grain and straw and increase of citrate-soluble P_2O_5, water-soluble SO_3 and acidity in the composts is readily apparent. With the exception of seed production on the silt loam, it appears that sulfur alone was as effective in promoting crop yields as its combination with rock-phosphate. Addition of rock-phosphate and sulfur to the manure at the time of applying the latter led to better yields than those obtained from the rock-phosphate compost. It will be observed that the use of calcium sulfate and mono-calcium phosphate was attended by relatively poor yields. It seems probable that this was due to excessive development of soil acidity from the latter salt.

Another composting experiment with manure was arranged on the same scale of magnitude as that just reported. Considering the relatively low development of acidity from sulfur in the previous test as compared with the results of Lipman and his coworkers, this test was conducted over a longer period than the preceding one. Furthermore, an additional compost of rock-phosphate and sulfur was conducted with weekly mixing, to promote aeration. As before, rock-phosphate and sulfur were used in the proportion of 3 to 1, as early used by Lipman (8, p. 513), and the proportions between these and the dry matter of the manure were equal to Lipman's proportions between these and the soils used. The sulfur was passed through an 80-mesh and the rock-phosphate through a 60-mesh sieve. A moisture content of 76.4 per cent was found in the mixture of fresh horse and cow manures. Portions of compost materials were prepared as follows:

Jar 1. 4540 gm. manure.
Jar 2. 4540 gm. manure (receiving ultimately 300 gm. acid-phosphate).
Jar 3. 4540 gm. manure (receiving ultimately 150 gm. rock-phosphate and 50 gm. sulfur).
Jar 4. 4540 gm. manure and 150 gm. rock-phosphate.
Jar 5. 4540 gm. manure and 50 gm. sulfur.
Jar 6. 4540 gm. manure and 150 gm. rock-phosphate and 50 gm. sulfur.
Jar 7. 4540 gm. manure and 150 gm. rock-phosphate and 50 gm. sulfur, to be mixed weekly for aeration.

After standing at room temperature for 97 days, and again after 129 days, the manures and composts were mixed and sampled for moisture determinations. On the latter date they were also subjected to the usual chemical analysis, the delayed additions now having been made. Lipman's analytical methods were used (8, p. 511). Fifty grams of the sample were extracted for determining citrate-soluble P_2O_5 in the material from jars 1 and 2, but 100 gm. were used in all other cases. The data obtained are presented in table 6.

Inspection of the data shows that the percentage of total P_2O_5 which became soluble in ammonium-citrate solution where rock-phosphate was composted was decidedly increased by the supplementary application of sulfur. While the acidity was reduced in the latter compost by frequent mixing, the solubility of the P_2O_5 was decidedly enhanced thereby. If multiplied by 5, to bring them

to approximate equivalence with Lipman's data (basis of 120 gm. dry matter), the maximum acidities of the sulfur-treated composts are about equal to that found by Lipman, McLean and Lint in their compost of garden soil with rock-phosphate and sulfur after a like period of 18 weeks (1, table 2), but they found twice as great acidity where sulfur was composted alone. The use of sulfur alone did not promote solubility of the P_2O_5 as it did in the preceding experiment. These results agree with the observation of Lipman and his associates (8, p. 518) that sulfofication gains intensity after about 18 weeks.

The availability of P_2O_5 in these composts was tested with sand cultures of barley, following the method of Lipman and McLean (7). Sand of unusual purity supplied by the Ottawa Silica Company of Illinois was used, after thorough washing with water. It consisted of spherical particles screening be-

TABLE 6

Composition of fermented manure and composts of manure with sulfur and phosphates

COMPOST MATERIALS	DRY MATTER		LOSS OF ORGANIC MATTER	ACIDITY ON BASIS OF 100 GM. OF ORIGINAL MANURE	CITRATE-SOLUBLE P_2O_5	TOTAL P_2O_5	SOLUBILITY OF P_2O_5
	96 days	129 days					
	per cent	*per cent*	*per cent*	*cc. 0.02 N alk.*	*per cent*	*per cent*	*per cent*
Fermented manure	31.1	44.3	53.7	28	1.50	2.72	55.2
Fermented manure and acid-phosphate	34.2	45.2	49.4	488	5.34	6.61	80.8
Fermented manure, rock-phosphate and sulfur	24.9	31.4	48.9	27	1.19	7.48	15.9
Compost of manure and rock-phosphate	28.3	33.0	52.0	24	1.26	8.05	15.7
Compost of manure and sulfur	31.2	37.6	23.7	426	0.89	1.65	53.9
Compost of manure, rock-phosphate and sulfur	37.3	45.6	18.6	464	1.24	5.14	24.3
Compost of manure, rock-phosphate and sulfur, mixed weekly	47.3	61.5	18.8	259	1.48	4.87	30.4

tween 60 and 70-mesh, and had a water-holding capacity of 23.4 per cent. Portions of 3.5 kgm. were placed in glazed stoneware jars of 0.5-gallon capacity. The following salt mixture was then incorporated with each portion of sand:

3.9 gm. $CaCO_3$
0.8 gm. K_2SO_4
0.4 gm. $MgSO_4 . 7H_2O$
0.1 gm. $Fe_2(SO_4)_3$

$NaNO_3$ was applied later to the extent of 1.2 gm. per jar, 0.4 gm. being added in solution soon after the seedlings appeared and the rest 11 days later. Manurial additions of the composts equivalent to 1 gm. of rock-phosphate in

composts containing the latter were tilled into the surface sand of the culture jars. A pedigreed strain of barley was sown and distilled water was applied to the extent of 36 per cent of the full holding capacity of the sand. After the seedlings were well established the cultures were reduced to 6 plants per jar and the water plane was increased to 60 per cent of saturation. The crops were photographed (plate 3) after 82 days of growth and were harvested one week later. Data of the manurial treatments and yields of dry matter appear in table 7. The results also appear graphically in figure 2.

It will be noted that direct use of rock-phosphate and sulfur with the fermented manure produced as good yields of seed as the usual composting treatment of these materials. There was an increased yield from the aerated complete compost, but this increase was not proportionate with the increase of

TABLE 7

Yields of barley from fertilized sand cultures supplemented by composts

JAR NUMBER	MANURIAL ADDITION		SEED	STRAW
		gm.	gm.	gm.
1	Fermented manure	9.10	3.2	10.4
2	Fermented manure	9.10	2.7	10.2
3	Fermented manure 9.73 gm., and acid-phosphate	2.00	5.9	12.0
4	Fermented manure 9.73 gm., and acid-phosphate	2.00	6.0	12.7
5	Fermented manure 14 gm., rock-phosphate 1.0 gm., and sulfur	0.33	5.8	11.6
6	Fermented manure 14 gm., rock-phosphate 1.0 gm., and sulfur	0.33	5.6	12.3
7	Compost of manure and rock-phosphate	16.20	3.3	9.9
8	Compost of manure and rock-phosphate	16.20	1.6	8.5
9	Compost of manure and sulfur	17.33	5.9	13.4
10	Compost of manure and sulfur	17.33	5.8	12.5
11	Compost of manure, rock-phosphate and sulfur	17.57	5.5	13.1
12	Compost of manure, rock-phosphate and sulfur	17.57	5.8	12.2
13	As above, but aerated by weekly mixing	13.13	6.8	13.2
14	As above, but aerated by weekly mixing	13.13	5.9	12.6

citrate-soluble P_2O_5. We believe these results indicate that available P_2O_5 produced by composting is subjected to competitive biological and other influences in the soil, which may alter its condition before absorption by the plant occurs. The results are very favorable for the complete composts, in so far as they produced yields equal to those derived from the use of acid-phosphate. As in the case of sandy loam of the previous culture experiment, sulfur alone, as a composting addition, has shown remarkable efficiency in grain production. It was as effective as any of the other manurial treatments, unless exception be made of the questionable superiority of the aerated compost. This predominant effect of sulfur raises the question as to whether it functions under such conditions by liberating available P_2O_5 from the manure, supplementing the efficiency of small supplies of the latter, or by acting directly as a nutrient,

after oxidation. If the first of these conditions obtains it appears, in view of the results of analysis, that it may have been brought about after the compost was applied to the barley cultures in this experiment. Exact comparison of the P_2O_5 absorbed by the cultures which received sulfur compost with that absorbed where manure alone was applied would be of interest, but material was not reserved for these determinations. Computation shows the application of citrate-soluble P_2O_5 per culture for the vertical order of manurial additions of table 7 to have been as follows: 60 mgm., 342 mgm., 68 mgm., 68 mgm., 58 mgm., 99 mgm., and 119 mgm. Assuming an average of 0.8 per cent P_2O_5 in barley seed, the absorption of this constituent by this part of the plants would have been only 24 mgm. in the average manured culture and 46 mgm. in the one which received sulfur compost. According to Pember (9) the minimum P_2O_5 requirement of 6 barley plants, as determined by the water-culture method, is 45 mgm. These data indicate that all the composts

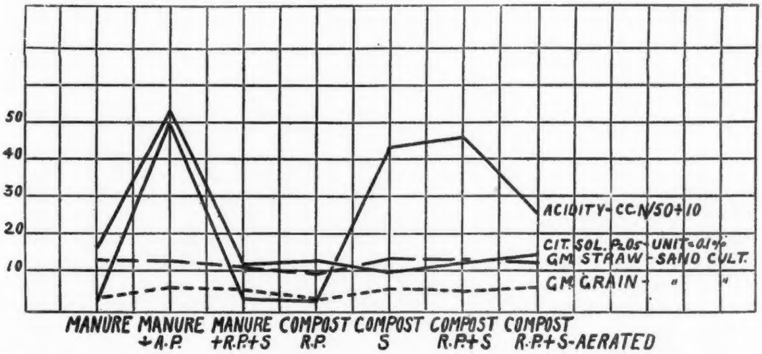

FIG. 2. COMPARISON OF YIELD (SAND CULTURES) WITH COMPOSITION OF 18-WEEK COMPOSTS

as applied conveyed liberal supplies of available P_2O_5. We are inclined to believe, however, that the manure applied alone and the compost containing only rock-phosphate suffered reversion of this form of P_2O_5 in the presence of $CaCO_3$ in the culture jars. The same effect is also likely to arise, of course, from the natural alkaline fermentation of manure. Hence, it appears quite possible that the beneficial nutrient effects of sulfur composts already pointed out are due to the production and maintenance of an adequate supply of available P_2O_5 from the manure used, as a result of acidity arising from sulfofication.

After samples of these composts which had been dried at 98°C. had been kept about one year they were analyzed for total sulfur, sulfate sulfur, sulfite and thiosulfate sulfur combined and elemental sulfur (CS_2 extraction). The analysis included the control manure, the compost with sulfur and the unaerated compost with sulfur and rock-phosphate. Less than 0.1 per cent of sulfite and thiosulfate sulfur occurred in any case. Of the total sulfur

of the manure only a trace was present as SO_3, while in the sulfur compost the latter formed 36.9 and in the complete compost 45.6 per cent of the total sulfur. The corresponding values for elemental sulfur were 0.0, 50.6 and 42.8 per cent of the total sulfur, respectively.

In field trials we have tested the efficiency of composts as compared with the direct application of rock-phosphate and sulfur with manure. The results are inconclusive, due, apparently, to the considerable reserve of P_2O_5 in Miami silt loam, to which soil the trials have been thus far confined. In the season of 1920, barley on plots 1 by 2 rods in dimensions gave slightly better yields of grain where the manurial treatment consisted of fermented manure, rock phosphate and sulfur than where the corresponding compost was applied. The applications were equivalent to 8 tons of fresh manure per acre, reinforced with 40 pounds of rock-phosphate and 13.7 pounds of sulfur per ton.

TABLE 8

Yields of air-dry matter from greenhouse crops to which sulfur was added with or without lime

FERTILIZER ADDITION	ON MIAMI SILT LOAM					ON PLAINFIELD SANDY LOAM
	Clover	Mustard		Rape	Turnip	Clover
		Seed	Straw			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nothing	55.0	2.7	44.0	19.3	13.9	61
10 gm. $CaCO_3$	54.1	2.8	49.0	13.0	11.8	102
0.5 gm. sulfur	70.5	4.0	42.5	21.8	16.2	54
0.5 gm. sulfur and 10 gm. $CaCO_3$	59.7	1.6	36.6	13.3	10.9	96
1.0 gm. sulfur	69.2	2.4	36.0	20.8	16.5	59
1.0 gm. sulfur and 10 gm. $CaCO_3$	60.2	3.2	32.0	11.6	15.5	102
3.0 gm. sulfur	69.2	4.5	46.5	22.4	14.7	52
3.0 gm. sulfur and 10 gm. $CaCO_3$	70.1	2.5	54.0	14.9	13.1	106
5.0 gm. sulfur	70.9	5.3	58.0	20.9	12.1	41
5.0 gm. sulfur and 10 gm. $CaCO_3$	63.5	1.8	44.2	14.1	10.5	96

Shedd (11) obtained beneficial effects on the yield by applying elemental sulfur to various crops in comparison with sulfates. Reimer and Tartar (9) have conducted similar comparisons with alfalfa in southern Oregon, with remarkable results from sulfur on the basic soils used. They consider an application of 40 pounds of sulfur yearly to be sufficient.

We have used elemental sulfur as a fertilizer in both greenhouse and field tests. In the former case plants were selected from species which absorb sulfur freely. Sulfur was applied in amounts ranging from 0.5 to 5 gm., either with or without 10 gm. of $CaCO_3$, per portion of soil. The latter consisted of either 15 kgm. of Miami silt loam or 20 kgm. of Plainfield sandy loam in cypress boxes. These additions are equivalent to 1 ton of limestone and 100 to 1000 pounds of sulfur per acre. The data of sulfur additions and yields are assembled in table 8.

Apparently the sandy loam was deficient in lime. The benefits from sulfur were generally considerable, but the rate of 100 pounds per acre was as efficient as the larger applications.

The use of sulfur on field plots of barley has given results which confirm the beneficial effect of the element, apart from application with rock-phosphate. Applications of the fertilizer materials were tilled into plots of 2 square rods area (1 by 2 rods) each on sandy silt loam in 1919. No farm manure had been applied to this land for four years. Barley was sown in 1919 and 1920, with no fertilizer applications the latter year. Table 9 contains the data of treatment and yields. The striking effect of lime in promoting maturation of the crop in 1919 is shown in plate 4. Heading out was completed several days earlier on the limed than on the unlimed plots.

TABLE 9

Yields of barley grain from plots treated with sulfur, with and without lime

PLOT NUMBER	FERTILIZER ADDITIONS OF 1919, PER ACRE	YIELD PER ACRE*	
		1919	1920
		bu.	*bu.*
27	Nothing	41.7	36.7
13	1000 lbs. marl	45.0	40.9
26	500 lbs. rock-phosphate	48.3	43.4
12	1000 lbs. marl and 500 lbs. rock-phosphate	47.5	38.4
23	500 lbs. rock-phosphate and 50 lbs. sulfur	43.4	35.0
11	1000 lbs. marl, 500 lbs. rock-phosphate and 50 lbs. sulfur	46.7	35.0
17	100 lbs. sulfur	54.2	50.0
16	1000 lbs. marl and 100 lbs. sulfur	55.0	50.8
24	500 lbs. rock-phosphate and 100 lbs. sulfur	45.8	35.0
14	1000 lbs. marl, 500 lbs. rock-phosphate and 100 lbs. sulfur	51.7	42.5
25	500 lbs. rock-phosphate and 300 lbs. sulfur	51.7	38.4
15	1000 lbs. marl, 500 lbs. rock-phosphate and 300 lbs. sulfur	47.5	43.4

* 48 lb. per bushel.

The beneficial effect of liming noted in the time of maturation is reflected also in the yields of barley. As regards the use of sulfur with rock-phosphate, 100 pounds per acre gave greater yields than either 50 or 300 pounds. However, in harmony with the results from sand cultures already presented, sulfur at the medium rate of application was as efficient alone as when supplemented with either rock-phosphate or rock-phosphate and lime.

As a practical consideration, the use of sulfur was profitable in this test. With the then current price of 8 cents per pound for sulfur, it appears from table 9 that a 100-pound application of the element produced a 26-bushel increase of barley over the yield from untreated soil in the two successive years, at a cost of 31 cents per bushel.

Although this effect of sulfur seems to indicate an important function of the element in rendering P_2O_5 available from latent forms in soils and composts,

the possibility must not be overlooked that it may function also in the form of oxidation products. These may be either stimulating intermediate forms or the more strictly nutrient sulfates ultimately formed. Reimer and Tartar (10, p. 35) emphasize the function as sulfates. The results of our determinations of forms of sulfur in the composts applied to sand cultures indicated little importance quantitatively for the intermediate products of oxidation.

In other field experiments on Miami silt loam, now in progress where steamed bone meal is used in conjunction with potassium salts and gypsum, no increase in yield of barley, either straw or grain, has been observed over a period of 7 years. These results are introduced here as evidence of probable lack of effect from sulfur as an oxidation product in the form of sulfates, in the sulfur test just reported. It should be remembered, nevertheless, that the sulfate requirement of cereals is low as compared with that of alfalfa, the crop used in the work of Reimer and Tartar.

As a further test of the importance of sulfates in the action of elemental sulfur, we have used the two in comparison, and also superimposed sulfur upon sulfates, in a greenhouse experiment.[4] Portions of 15 kgm. of Miami silt loam were placed in the galvanized iron boxes previously described. After incorporating the desired additions, with the exception of $NaNO_3$, the soils were sown to oats and watered to 20 per cent of their holding capacity. The seedlings were reduced to uniform numbers per culture, and the water plane was gradually raised to 60 per cent of saturation. It was intended to reduce gradually the water plane as maturation set in, but early in the period of seed formation the cultures became quite dry for a brief period. This condition, and the unusually low light intensity, even for the season of growth (October 16 to March 1), may account for the erratic yields of seed obtained from duplicate cultures. The usual care was taken to rotate the cultures as to position within the house. Growth was rapid and apparently normal, excepting a form of leaf burn where sulfur was superimposed upon sulfates, and uniformly slow filling of the seed.

The complete fertilizer consisted of 5 gm. $NaNO_3$ (applied in portions to the growing plants) 5.85 gm. KCl and 10 gm. $Ca_3(PO_4)_2$ culture. Lime was added in the form of 10 gm. $CaCO_3$ per culture. Sulfur, sodium sulfate and calcium sulfate were applied in equivalent amounts upon three planes, designated as "low," "medium" and "high." These applications were equivalent to 0.5, 1.4 and 4.2 gm. sulfur per culture or 33, 100 and 300 pounds per acre, respectively.

Inasmuch as the yields of seed were quite variable from cultures treated in duplicate, we shall neither present the data in full nor comment extensively upon them. The yields of straw gave consistent duplicate values, with the exception of the supplementary treatment of sulfur added to sulfates. For

[4] This experiment was performed by S. Lepkovsky, as also the determination of forms of sulfur in the composts applied to sand cultures.

the most part, the total seed yields from various treatments varied in much the same direction and order as the corresponding yields of straw.

As to general indications from this test, gypsum was superior to sulfur, whether or not lime was added. It was also superior to sulfate of sodium on the limed soil. This is in agreement with previous observations (13, p. 248). Liming depressed the efficiency of gypsum; moreover, all of the erratic seed yields occurred where lime was applied. It thus appears that an excess of lime on the soil may not only depress the availability of P_2O_5, but also interfere with the functions of sulfate of lime. Such interference as the latter might ensue if, as suggested by one of the writers (5, p. 437), gypsum functions in an important manner by combining the supply of sulfur with calcium in molecular form. Thus, an excess of Ca as $Ca(HCO_3)_2$ in the soil solution might depress the absorption of $CaSO_4$, and hence of sulfur, by the plant.

The best results from elemental sulfur attended the medium application of 100 pounds per acre. This rate, in view of agreement with previous tests, therefore seems to be fairly well established as optimal for Miami silt loam under our experimental conditions. Inasmuch as sulfur was ineffective when superimposed upon sulfates in this test, it might seem that the results lend support to the belief that the element functions largely through conversion to sulfates. It is quite possible, however, that the presence of sulfates interfered with the oxidation of sulfur or otherwise prevented its favorable effect upon the availability of P_2O_5.

It follows logically that the use of sulfur in field practice will sooner or later tend to such depletion of P_2O_5 as to necessitate the supplementary use of phosphate fertilizer. The same will be true of lime, and probably other soil constituents, to a greater or less degree.

SUMMARY

Soil composts with sulfur added developed much acidity in 32 weeks. The values were greater for sulfur alone than where rock-phosphate also was added. Dissociated acid formed but a small portion of the total acid.

Composts of sulfur with horse manure showed appreciable increase in acidity, but no increase of citrate-soluble P_2O_5, after 15 weeks.

Sulfur in composts with 4.54 kgm. of manure decreased the loss of organic matter by fermentation. Increased bacterial counts in these composts were maintained to the conclusion of 12.5 weeks. Acidity doubled in the rock-phosphate and sulfur compost over this period, but was unchanged after 4.5 weeks. The variations of water-soluble SO_3 were in the same direction as those of acidity, increasing where sulfur was added and decreasing in the other cases. Citrate-soluble P_2O_5 approximately doubled where sulfur was added, the percentage being based upon the common basis of original dry matter of the composts. In the other cases the percentage of this constituent decreased approximately one-half. Yields of oats from soil cultures to which these com-

posts were applied agreed generally with the results of analysis of the latter, producing the greatest yields where sulfur had been applied. On Plainfield sandy loam the yield of seed where sulfur compost was applied was as great as where the corresponding treatment included rock-phosphate. Increased yields of oats were obtained also from the application of rock-phosphate and sulfur with fermented manure.

Similar composts which fermented 18.5 weeks showed 60 per cent greater availability of P_2O_5 (solubility in ammonium-citrate solution), by the addition of sulfur to rock-phosphate. When aerated by weekly mixing the corresponding increase of available P_2O_5 was 90 per cent, although the total acidity was decreased thereby. The total acidity developed in the complete compost was nearly as great as that resulting from an equivalent addition of acid-phosphate to the fermented manure. In this experiment, the use of sulfur alone did not increase the proportion of citrate-soluble P_2O_5 in the manure. Application of these fermented manures and composts to sand cultures of barley led to equal yields from the rock-phosphate and sulfur compost and a corresponding portion of fermented manure supplemented by acid-phosphate. These yields were not superior to those from sulfur compost and manure reinforced by rock-phosphate and sulfur.

The process of sulfofication is inactive after 12 weeks, but becomes very active after 18 weeks.

In greenhouse trials on Miami silt loam and Plainfield sandy loam with clover and cruciferae, sulfur increased growth on the former soil. One hundred pounds of sulfur per acre was as effective as more.

Barley on field plots of sandy silt loam apparently in need of lime produced increased yields of seed by the application of sulfur. Sulfur alone was as effective as its combinations with marl and rock-phosphate. One hundred pounds of sulfur per acre was as effective as 50 or 300 pounds.

Sulfate of lime produced better yields of oats upon Miami silt loam than equivalent amounts of sulfate of soda or sulfur, when these supplemented the usual complete fertilizer in greenhouse cultures. Liming depressed the efficiency of sulfate of lime under these conditions. One hundred pounds of elemental sulfur was more effective than either one-third or three times as much. Benefits from elemental sulfur were not apparent when it was superimposed upon the sulfates for application.

It appears probable that sulfur functions as a fertilizer both by oxidation to the nutrient SO_3 and by producing, through oxidation, an acid condition favorable to the production of available P_2O_5. These processes occur in composts of sulfur and rock-phosphate. They also, doubtless, continue when the compost materials are tilled into the soil.

It remains to be proved whether the efficiency of sulfur is any greater when it is composted with rock-phosphate and manure than when these materials are added simultaneously to the soil.

Adequate consideration of the use of sulfur as a fertilizer must recognize its tendency to deplete the stock of CaO, P_2O_5 and other soil constitutuents.

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PLATE 1

Oats on Miami Silt Loam

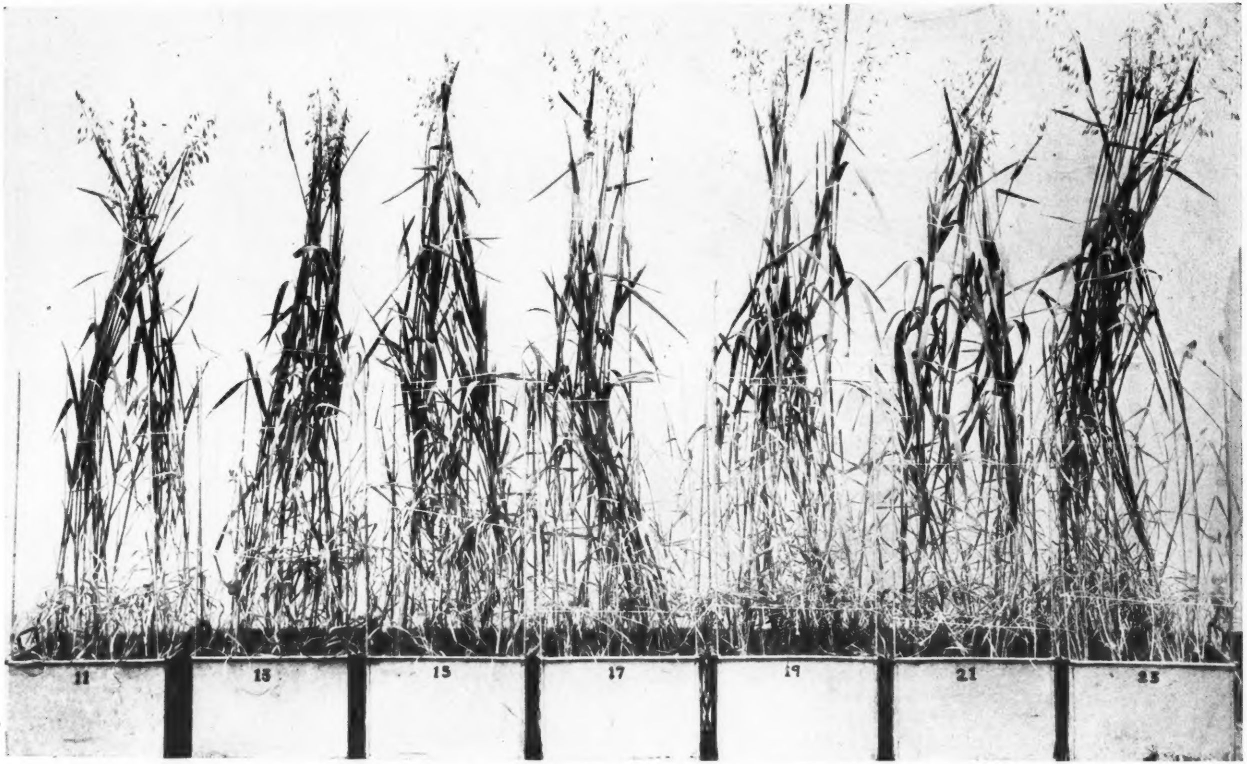
In each case, culture 1 of table 5

Untreated | Manure | Manure, $Ca(H_2PO_4)_2$ and $CaSO_4$ | Manure, rock-phosphate and sulfur | Compost rock-phosphate and sulfur | Compost rock-phosphate | Compost sulfur

PLATE 2

OATS ON PLAINFIELD SANDY LOAM

In each case, culture 1 of table 5



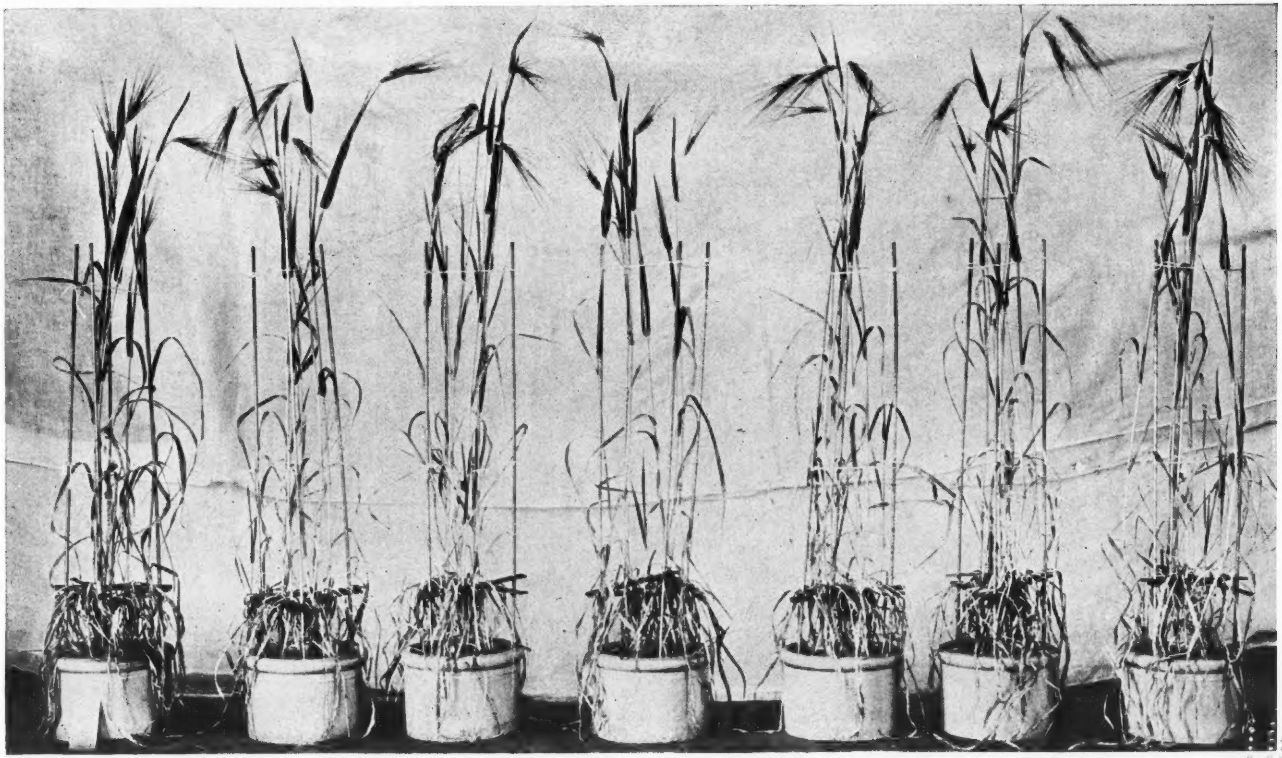
Untreated	Manure	Manure, $Ca(H_2PO_4)_2$ and $CaSO_4$	Manure, rock-phosphate and sulfur	Compost rock-phosphate and sulfur	Compost rock-phosphate	Compost sulfur

PLATE 3

Barley on Sand with Addition of a Phosphorus-Free Salt Mixture

Odd-numbered jars of table 7

PLATE 3



Manure | Manure and acid-phosphate | Manure, rock-phosphate and sulfur | Compost rock-phosphate | Compost sulfur | Compost rock-phosphate and sulfur | Compost rock-phosphate and sulfur aerated

PLATE 4

Maturation Difference in Barley of Sulfur Trials, 1919

Left tier, limed; right tier, unlimed

A COMPARISON OF INOCULATED AND UNINOCULATED SULFUR FOR THE CONTROL OF POTATO SCAB[1]

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INTRODUCTION

It has long been known that the presence or absence of the potato scab organism (*Actinomyces chromogenus* Gasperini) is determined largely by the soil reaction. As a result of this knowledge studies on control measures have been based to a large extent on determining some practical method whereby the soil reaction can be so changed as to produce conditions unfavorable for the development of scab. Various substances have been tested in this connection and of these sulfur has been found to be the most promising. In its use, however, much contradictory evidence has resulted. In some instances very good control was obtained while in others it proved practically worthless. In a previous paper (8) the statement was made that some of the reported failures of sulfur to control scab may have resulted from the fact that the sulfur was not oxidized. It is recognized that environmental conditions play an important rôle in the oxidation of sulfur but the presence of sulfofying micro-organisms would appear to be of even greater importance. Boullanger and Dugardin (1) found that the effect of sulfur on crop yields was not as marked on sterilized as it was on unsterilized soils. They conclude that this difference resulted from the fact that the oxidation of the sulfur was brought about by bacterial activities. As a result of adding sulfur to sterilized and unsterilized soil Demolon (4) found very little sulfur to have been oxidized in the sterilized soil while a considerable amount was oxidized in that which was unsterilized. Brown and Kellogg (2) found that each soil has a definite sulfofying power. They also found that while the formation of a small amount of sulfates was brought about by chemical action, the presence of bacteria is essential. Lipman and his co-workers (6) have shown that elemental sulfur is readily oxidized in soils containing sulfofying bacteria. In a later paper (7) as a result of a comparison of untreated soils with soils that had been sterilized and inoculated, and others unsterilized and uninoculated, they demonstrated the biological factor to be influential in the oxidation of sulfur.

From this brief summary of a portion of the literature on the oxidation of sulfur in soils the importance of the presence of sulfofying organisms is appar-

[1] Paper No. 12, of the Technical Series, New Jersey Agricultural Experiment Stations, Department of Plant Pathology.

ent. It is very probable that the lack of these organisms in the soil might determine to a large extent the success or failure of sulfur to control scab. The experiments herein reported were conducted to determine to what extent this might be true.

EXPERIMENTAL

In the experiments here reported each treated plot was adjoined by an untreated check plot and the size of the plots was made large enough to reduce as far as possible the error arising from soil variations. Except for the sulfur applications all plots in an experiment were treated alike as regards fertilization and cultivation.

Inoculated and uninoculated commercial flour sulfur was used. Inoculation was effected by means of a thorough mixing of the commercial flour with 1 per cent of soil from a compost heap known to be well supplied with the sulfur-oxidizing organisms. The sulfur applications were made with a grain drill just after harrowing and just before planting. This method proved very satisfactory, as it not only insured uniform distribution but at the same time worked the sulfur thoroughly into the upper several inches of soil.

The experiments were conducted with the Irish Cobbler, a variety known to scab very severely. When harvested, the crop was divided into three classes, namely, clean, salable and unsalable scabby. The first class was made up of all tubers free from scab. The tubers in the second class showed only a moderate infection while those in the last class were for most part covered with scab lesions.

Soil samples were taken before the sulfur applications were made and again at digging time. In taking the soil samples borings were made to a depth of 6½ inches at intervals of approximately 15 feet. The hydrogen-ion concentration of water extracts of the soil samples was determined colorimetrically following the method devised by Gillespie (5). The indicators used were those recommended by Clark and Lubs (3). In preparing the water extracts of the soil samples to be tested, the method followed was essentially the same as described in the previous paper (8).

The tables and diagrams show the yield per acre as well as the relation of clean, salable and unsalable scabby tubers to the hydrogen-ion exponent. The data given are averages obtained from at least three replications of each treatment and from four check plots.

Experiment I

Section A. The soil on which this experiment was conducted is a Sassafras loam. In 1915 an application of ground limestone was made at the rate of 3 tons per acre. Following this treatment the field was in grass in 1916 and 1917, and in potatoes in 1918 and 1919. In the latter year there was a uniform infection of scab over the entire field, a greater part of the crop being unsalable.

The field was divided into two parts that may here be designated as sections A and B. On the former a comparison was made of 600-pound applications of inoculated and uninoculated sulfur. On section B the amount employed was reduced to 300 pounds. These two sections will be discussed in turn.

The effect of the 600-pound application on the total yield and the number of clean and scabby potatoes is shown in table 1. It will be observed that the plots treated with both the inoculated and the uninoculated sulfur showed increases in yield as compared with the check plots. This increase can doubtless be explained in part on the ground that over 90 per cent of the tubers on the check plots were very severely scabbed, necessarily resulting in considerable decrease in weight. The severity of the attack is plainly evident from the appended photographs. These photographs are made up to show the relative proportion of clean, salable and unsalable scabby tubers from the various plots.

With both the uninoculated and the inoculated sulfur there was a marked decrease in unsalable scabby potatoes, amounting to 42.4 per cent for the former and 69.2 per cent for the latter. It is apparent that the greatest decrease resulted from the use of the inoculated sulfur. The benefit derived from the use of the latter is more clearly brought out when the number of tubers free from scab is considered. On the untreated plots none were clean as compared with 10.9 per cent for the plots treated with the uninoculated and 46.9 per cent for those receiving the inoculated sulfur.

TABLE 1

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSALABLE SCABBY	pH VALUES
	bushels	*per cent*	*per cent*	*per cent*	
*Check	195.0	0	6.4	93.6	7.06
*600 lbs. uninoculated sulfur per acre	229.0	10.9	37.7	51.4	6.23
*600 lbs. inoculated sulfur per acre	249.4	46.9	28.7	24.4	5.60

* Average of 3 plots.

In the diagrams of figure 1 are shown the relation of the hydrogen-ion exponent to the per cent of clean, salable and unsalable scabby potatoes. The initial hydrogen-ion exponent of this soil was 6.8 as compared with 7.05 for the check plots at the time of harvesting. This difference may be accounted for by the fact that the latter figure represents the composite soil sample from the check plots while the former represents that of the entire experimental area. It is evident however, that little change in exponent values occurred during the growing season. It will be seen from the diagrams that the plots receiving the 600-pound application of uninoculated sulfur showed a decrease in exponent values amounting to 0.83 as compared with 1.45 for those receiving the inoculated sulfur. With each decrease in the value of the hydrogen-ion

exponents below that of the check plots, a corresponding decrease occurred in the per cent of scabby tubers.

Section B. In this part of the field the scab infection was not as severe as in section A but there was a uniform distribution of the scab organism, a

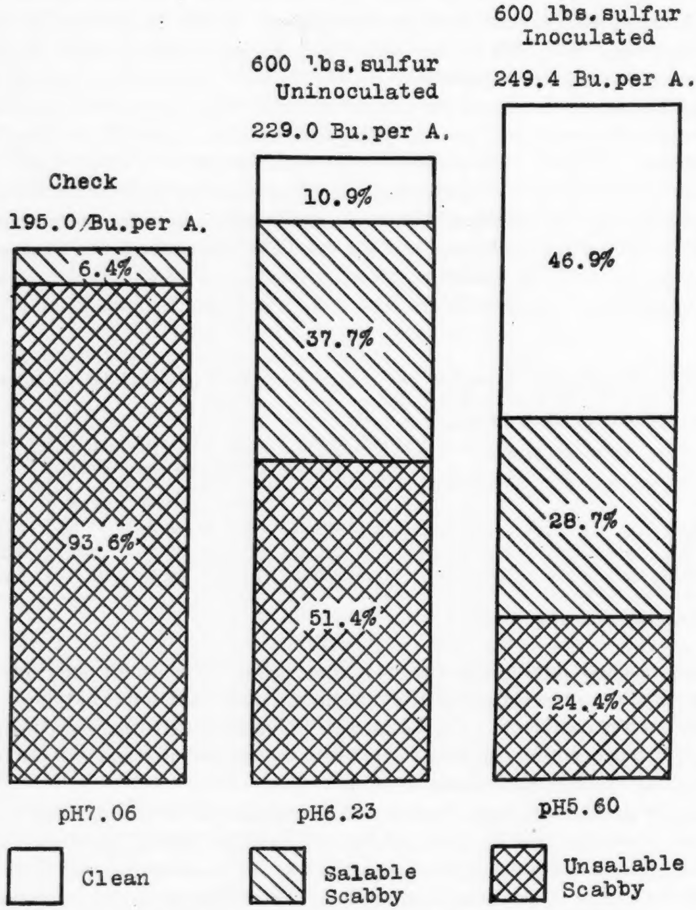

FIG. 1. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 600 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

great part of the 1919 crop being severely scabbed. Applications of inoculated and uninoculated sulfur were made at the rate of 300 pounds per acre. The results are presented in table 2. The data of this table indicate a marked increase in yield for the treated over the check plots, amounting to 71.2 bushels

for the plots treated with the uninoculated and 105.6 bushels for those treated with the inoculated sulfur. It is doubtful if this increase can be ascribed to the sulfur treatment alone. In this part of the field the yield became progressively better away from the check plots so that the increases in yield noted may be attributed for the most part to soil differences.

In this as in the preceding section the plots receiving the sulfur treatments showed a lower percentage of scabby tubers than the untreated plots. The differences noted are by no means as great, however. Of the total yield from the untreated plots 58.3 per cent were unsalable scabby while the plots treated with the uninoculated and those receiving the inoculated sulfur gave a total yield of which 57.4 per cent and 29.4 per cent, respectively, were unsalable.

The relation of hydrogen-ion exponents to the per cent of clean, salable and unsalable scabby tubers is shown in the diagrams of figure 2. The initial exponent value of these soils was 6.8. At the time of harvesting, the average hydrogen-ion exponent values of soil samples taken from the check plots was 6.6 while the corresponding exponent values for the samples from the plots

TABLE 2

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSALABLE SCABBY	pH VALUES
	bushels	*per cent*	*per cent*	*per cent*	
*Check	183.1	9.4	32.3	58.3	6.6
*300 lbs. uninoculated sulfur per acre	254.3	13.1	29.5	57.4	6.3
*300 lbs. inoculated sulfur per acre	288.7	34.1	36.5	29.4	6.0

* Average of 3 plots.

treated with the uninoculated and those treated with the inoculated sulfur were 6.3 and 6.0, respectively. With the decrease in exponent values there was a corresponding decrease in the number of scabby tubers.

A comparison of the data included in tables 1 and 2 with their corresponding figures shows that the 600-pound applications gave much better control of scab than the 300-pound applications, this being true for both the inoculated and the uninoculated sulfur. This is apparent from the figures given in table 3, which represent the percentage of increase or decrease of clean, salable and unsalable scabby potatoes, as well as the differences in exponent values, of plots treated with inoculated and uninoculated sulfur as compared with their corresponding check plots. The data given here are in accord with those previously published (8) which showed that where the hydrogen-ion concentration of water extracts of soil samples taken before the sulfur applications were made was 5.8 or less, lighter applications (300 to 600 lbs.) gave approximately as good control of scab as heavier applications (700 to 1200 lbs.). Where the initial exponent was greater than 6.0 the heavier applications gave the better

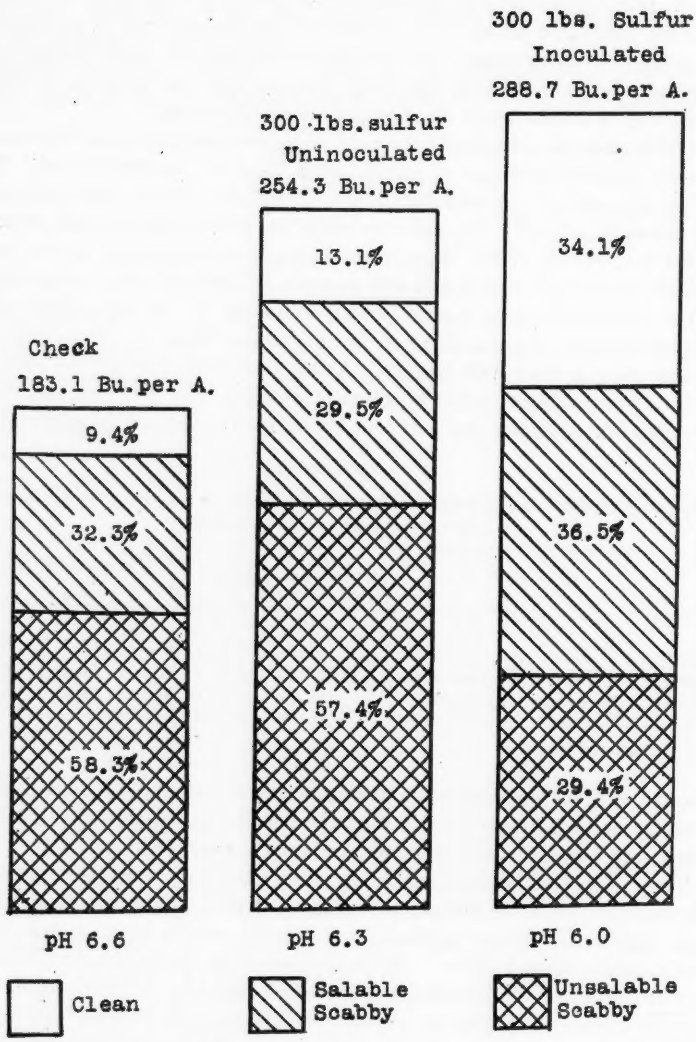

FIG. 2. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 300 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

control. From the results here reported it is evident that the 300-pound application was not sufficient to produce the acidity necessary to inhibit the growth of the scab organism. It is also apparent that with an exponent value as great

as that indicated for the check plots in the first experiment, namely 7.03, the amount of sulfur used should be in excess of 600 pounds in order that a greater part of the crop be rendered free from scab.

It will be observed from table 3 that the 300 and 600-pound applications of inoculated sulfur gave a greater decrease in exponent values compared with that of the corresponding checks than did similar amounts of uninoculated sulfur. With the decrease in exponent values there was a decrease in the number of unsalable scabby tubers with a corresponding increase in the two classes made up of salable potatoes. It is interesting to note from the table that the decrease in exponent values indicated for the plots treated with 300 pounds of inoculated sulfur was nearly as large as for the plots receiving the 600-pound application of uninoculated sulfur. The latter showed a reduction of 42.2 per cent in the number of unsalable scabby tubers as compared with 28.9 per cent for the former, a difference of only 13.3 per cent in favor of the heavier application. Greater differences resulted from the use of 300 pounds of inoculated and uninoculated sulfur; the inoculated showed a decrease of 28.9 per cent in

TABLE 3

Percentage of increase or decrease of clean, salable and unsalable scabby potatoes and differences in exponent values of plots treated with inoculated and uninoculated sulfur as compared with their corresponding check plots

TREATMENT	CLEAN TUBERS	SALABLE SCABBY	UNSALABLE SCABBY	DIFFERENCE IN pH VALUES
	per cent	*per cent*	*per cent*	
300 lbs. uninoculated sulfur per acre	+3.7	−2.8	−0.9	−0.30
300 lbs. inoculated sulfur per acre	+24.7	+4.2	−28.9	−0.60
600 lbs. uninoculated sulfur per acre	+10.9	+31.3	−42.2	−0.83
600 lbs. inoculated sulfur per acre	+46.9	+22.3	−69.2	−1.46

the number of unsalable potatoes as compared with 0.9 per cent for the uninoculated sulfur, a difference of 28 per cent in favor of the inoculation. The plots treated with 600 pounds of inoculated sulfur showed 27 per cent fewer unsalable scabby potatoes than the plots receiving 600 pounds of uninoculated sulfur. From the data here presented it is evident that the presence of sulfofying organisms not only tends to make the oxidation of sulfur more certain but as a result of this fact smaller quantities of the inoculated sulfur may be used than of the uninoculated to obtain the same control of scab.

Experiment II

The soil on which this experiment was conducted is a Penn loam that had not been planted to potatoes for a number of years. To insure the presence of the scab organism in the soil scabby seed was planted. This fact may account for the very low yields recorded, less than 100 bushels per acre. In view of the fact, however, that the primary purpose of the work was to determine the effect of the sulfur on scab control the low yields cannot be considered

a serious fault since each plot was affected alike. In this experiment sulfur was applied at the rate of 900 pounds of the inoculated and the uninoculated per acre. The results are given in table 4.

On the basis of total yield the plots treated with the uninoculated sulfur showed a decrease of 17.8 bushels as compared with the check plots. The plots treated with the inoculated sulfur showed a slight increase. The soil on which this work was conducted was very uniform and while the crop showed considerable scab the infection was by no means as severe as in the first experiment, very few tubers being rendered unsalable. This, no doubt, accounts for the fact that the striking differences in yield noted in the other experiments were not obtained here. As in the preceding experiments, there was a marked reduction of unsalable scabby tubers on the plots treated with inoculated sulfur with a much smaller reduction in the plots treated with the uninoculated sulfur. The increase in the per cent of clean potatoes was likewise greater for the inoculated sulfur, amounting to 38.2 as compared with 26.1 for the uninoculated sulfur.

TABLE 4

Influence of applications of inoculated and uninoculated sulfur on total yield, per cent of scabby tubers and hydrogen-ion concentration

TREATMENT	TOTAL YIELD PER ACRE	CLEAN TUBERS	SCABBY BUT SALABLE	UNSALABLE SCABBY	pH VALUES
	bushels	*per cent*	*per cent*	*per cent*	
*Check	78.0	43.2	34.4	22.4	6.6
*900 lbs. uninoculated sulfur per acre	60.2	69.3	15.4	15.3	4.6
*900 lbs. inoculated sulfur per acre	78.2	81.4	17.1	1.5	4.7

* Average of 4 plots.

The relation of the hydrogen-ion exponents of the soil samples from the plots in this experiment to the per cent of clean, salable and unsalable scabby tubers is shown in the diagrams of figure 3. Soil samples were taken of all the plots before the sulfur applications were made, the mean value of all the determinations being 6.61. The exponents of the check plots at the time of harvesting was 6.6. The values of the hydrogen-ion exponents of the soil samples corresponding to the two treatments show a considerable decrease over those of the check plots, being 4.6 for the plots receiving the uninoculated sulfur and 4.7 for those treated with the inoculated sulfur. While the exponent values for the two treatments are approximately identical, it will be seen from the diagrams that the number of unsalable tubers was reduced from 15.3 per cent recorded for the plots treated with uninoculated sulfur to 1.5 per cent, for those receiving the inoculated sulfur. On the basis of clean potatoes the latter showed an increase of 38.2 per cent over the checks as compared with 26.1 per cent for the uninoculated sulfur. It will be observed that the differences in scab control obtained in this experiment from the use of inoculated and uninoculated sulfur are by no means as great as in the first two experi-

ments reported. This may possibly be explained on the grounds that the plots in this experiment were smaller than in the others and their arrangement in the field was such that soil from one plot might have easily been carried to another in cultivation. In this way the plots treated with the uninoculated sulfur may have been inoculated at some time during the growing season. This would account for the fact that even with identical exponent values at digging time scab control was better on the plots receiving the inoculated sulfur than on those treated with the uninoculated sulfur. The former doubt-

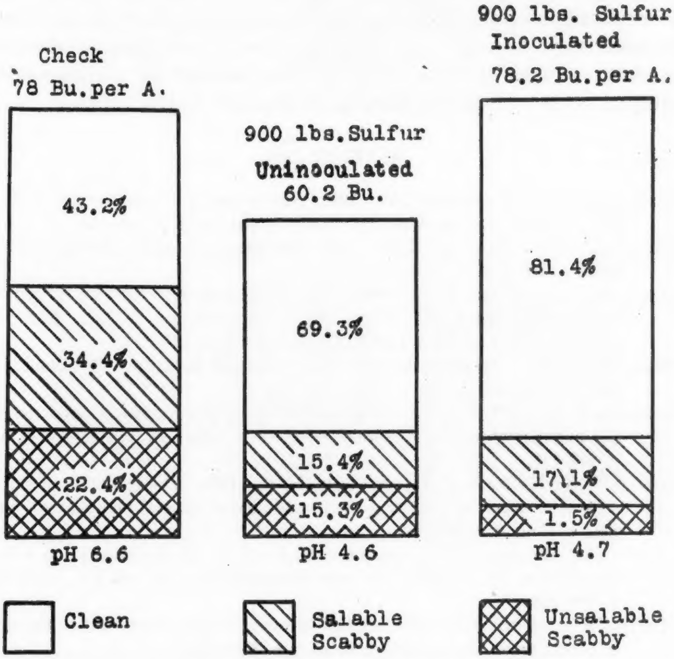

FIG. 3. DIAGRAMS SHOWING THE RELATION OF SULFUR TREATMENTS OF 900 POUNDS TO THE ACRE TO HYDROGEN-ION CONCENTRATION AND TO THE PER CENT OF CLEAN, SALABLE AND UNSALABLE SCABBY TUBERS

less oxidized earlier in the season bringing about a reduction in exponent values which resulted in the prevention of early infection. The latter, being inoculated at a later date, was not oxidized in time to prevent this early infection but was able to produce the same acidity by the time the crop was dug.

SUMMARY

1. The addition of sulfur to soil usually leads to an increase in soil acidity due largely to the oxidation of the sulfur by sulfofying micro-organisms. Where

these organisms are absent it is necessary that they be supplied in order that the sulfur be oxidized.

2. On the soils on which these experiments were conducted the use of sulfur inoculated with the sulfofying organisms gave better control of scab than similar amounts of uninoculated sulfur.

3. In addition to the difference in control the indications are that smaller amounts of inoculated than of uninoculated sulfur may be used to obtain the same results.

4. Hydrogen-ion exponent values of soil samples taken from plots treated with inoculated and from those treated with uninoculated sulfur were considerably lower than corresponding exponent values of soil samples taken from check plots. In most instances this increase in acidity was accompanied by a corresponding decrease in the number of unsalable scabby tubers.

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PLATE 1

Fig. 1. Tubers from check plots.
Fig. 2. Tubers from plots treated with 600 pounds uninoculated sulfur.
Fig. 3. Tubers from plots treated with 600 pounds inoculated sulfur.

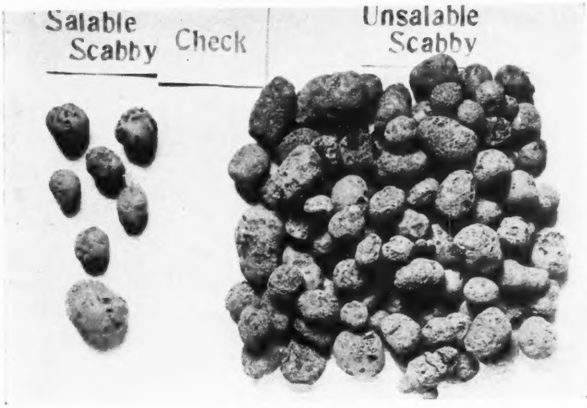

FIG. 1.

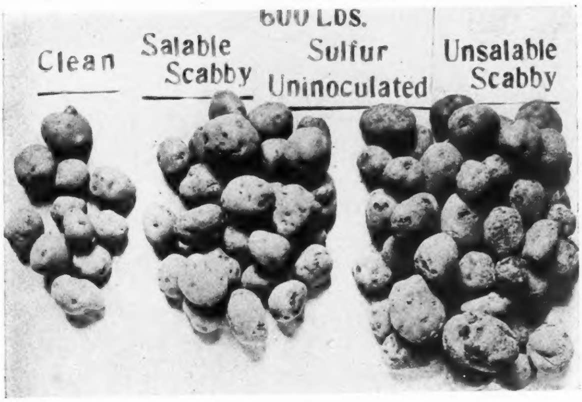

FIG. 2.

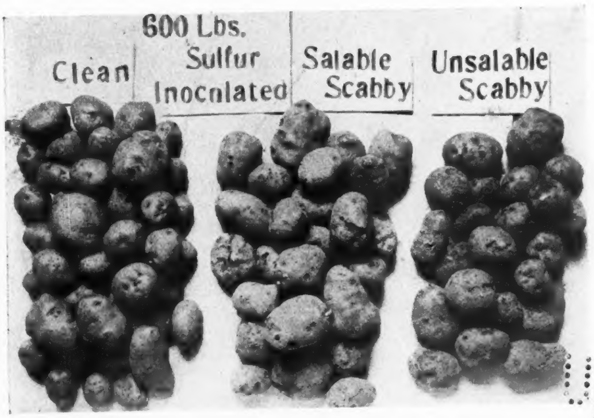

FIG. 3.